







# Contents

## Original Articles

- Andersen J B The Effect of Diuretics in Late Pregnancy on the Newborn Infant 639
- Aperia A, Berg U and Broberger O Renal Function during Hypoxemia and Water Diuresis in Children with Recurrent Urinary Tract Infections 605
- Aurora A L, Tanaya O P and Gupta D N Bladder Stone Disease of Childhood I An Epidemiological Study 171
- Aurora A L, Tanaya O P and Gupta D N Bladder Stone Disease of Childhood II A Clinico-Pathological Study 345
- Bakken A F Bilirubin Excretion in Newborn Human Infants I Unconjugated Bilirubin as a Possible Trigger for Bilirubin Conjugation 143
- Bakken A F Bilirubin Excretion in Newborn Infants II Conjugated Bilirubin as a Possible Trigger for Bilirubin Excretion 153
- Bellóns A, Moragas A, Gallart-Catalá A and Berán N Respiratory Pathology in the Immediate Postnatal Period 497
- Barak Y Osteoblasts and Osteoclasts in Bone Marrow Aspirates from Children with Rickets 363
- Barnes R D, Bohlen N P and Holaday J Impaired Lymphocyte Transformation and Chromosomal Abnormalities in Fetal Gravidomatous Disease of Child blood 403
- Beckman O, Beckman L, Gochelson L and Lempery, L Acid Phosphatase Activity in the Synovial Fluid of Children with Juvenile Rheumatoid Arthritis 65
- Bergman B, Gennier G and Nilsson E Sodium Potassium and Water Content of Human Fetal and Maternal Plasma and Red Blood Cells 192
- Berg U, Aperia A, Broberger O, Ekensjö A and Ericson N O Relationship between Glomerular Filtration Rate and Radiological Appearance of the Renal Parenchyma in Children 1
- Berglund G Three Cases of Documented Intraventricular Hemorrhage 864
- Berkel I, Krenn, O and Sey B Jejunal Meconia in Intestinal Malrotation 58
- Bethke J A The Fatty Acid Composition of Depot Fat in Childhood I The Effect of Age, Sex and Site on Superficial Fat 405
- Bernhoff A, Hall K, Skjerve J and Werner I Primary Hyperparathyroidism in Children: Brief Review of the Literature and a Case Report 49
- Brodeur J, Gellman, A. and Kaza, W P The Renal Transport of Amino Acids in Unborn Infants with Phenylketonuria 41
- Carlsson, G and Järfing, B Cytomegalovirus Infections in Different Groups of Paediatric Patients 301
- Chadd M A, Gray O P and Joyner J W Gastric GIB in Leukemia Newborn with Spontaneous Cytotoxic
- Dahl G Chromosomal Conditions in Congenital Heart Disease 65
- Dahlqvist A, Lindberg, T, Mectrasse G and Åkerman M Intestinal Dyspepsia and Dysmaturations in Children with Malabsorption 621
- Dahlqvist A, Gustafson I and Madsen H A Patient with Hereditary Galactokinase Deficiency 669
- Douchetovska R, A M G, Sander P C van der Kelenburg G J, Stoop J W and Tiddens H A W M Serum Creatinine Values in Healthy Children 399
- Eklund H, Hedner U and Nilsson I M Fibromyositis in Newborns 33
- Eklund H, Kullander S and Kallen B Major and Minor Malformations in Newborns and Infants up to One Year of Age 297
- Eklund H, Hedner U and Anders B Fibromyositis in Human Fetus 368
- Ekström R Anthropometry in Privileged Ethiopian Preschool Children CNU Report No 33 157
- Ericson, N O, Hellström B, Nergårdh A and Rudhe U Unilateral Neurological Defect in Myelomeningocele with Normal Bladder Function Report on Two Cases 487
- Ericson N O, Hellström, B, Nergårdh A and Rudhe U Factors Promoting Urinary and Anal Continence in Children with Myelomeningocele 491
- Erkman M Salicylate Induced Fetal Damage during Late Pregnancy in Mice The Modifying Effect of Repeated Administration and Dosage 517
- Forsgren J, Pagan Y and Sarvergrin J Liver Function Studies and Biliary Tract Investigations in Meconium 539
- Feldman W, Drummond J N and Klein M Hypotonia Following Asphyxial Neonatorum 52
- Fjord Christensen M, Nielsen J A and Henriksen O Treatment of Cystinosis with a Diet Poor in Cysteine and Methionine 613
- Gabarro G, Volpato S and Vign V Diagnosis of Beta Thalassemia in the Newborn by Means of Hemoglobin Synthesis 523
- Gell O M, Dryer N M and Rodd B T Urinary Testosterone Excretion in Children with Congenital Adrenal Hyperplasia 137
- Gladman H, Bhatta C K and Krichbaum D M The Significance of the Deficiency State in Leishmaniasis 233
- Goedde H W, Langenbeck, U., Brackert, D, Keller W, Rottkonec T, Halvorsen, S, Kall R and Merton B Clinical and Biochemical Genetic Aspects of Intermittent Branched-Chain Ketosiduria Report of Two Scandinavian Families 83
- Hagberg, B and Westphal O Ataxia Syndromes in Congenital Hypothyroidism 303
- Hagberg B, Hansson, O., Liden, S and Nilsson K Familial Ataxic Dystonia with Deficient Cellular Immunity A New Clinical Entity 545
- Hamberger L., Fällsberg, G and Kildeberg, P The





- Stimulation in Normal Children and in Patients with Cystic Fibrosis 692
- Östervik I and Flåegstad L R. Poliovirus Antibody Status Three Years after Vaccination with Trivalent Oral Vaccine 536

## Review Articles

- Hagberg H and Sellenpää M. Benign Intracranial Hypertension (Pseudotumor Cerebri) Review and Report of 18 Cases 578
- Leonard H G. Sleep Studies in Infancy: Facts, Concepts and Significance 572
- Öster J and Nielsen A. Nuchal Nervi and Inter-ocular Telangiectases: Incidence in Danish School Children 416

## Case reports

- Achard R A. Recurrent Bacteremia Following Ampicillin Treatment of Hemophilus Influenzae Meningitis 711
- Autonheim I and Ström S. Congenital Atrial Flutter: Report of a Case 587
- Berglund C O and Nilsson K O. Neonatal Myomeres Caused by Salmonella Thompson 437
- Burby C P, Roman R C, Northway W H Jr, Davis Smith P, Dwyer W J and Brand M M. Rapid Growth and Centriolobular Emphysema after Stereotaxic Tracheitis and Continuous Exposure to Low Concentration of Oxygen 697
- Fjord Christensen M and Therkelsen A J. A Case of the XXXXX Chromosome Anomaly with Pteris Myeloid X Chromosomes and Diabetic Glucose Tolerance 706
- Giesel D B, Alexander F W and Seakins J W T. Abnormal Tyrosine Metabolism in Hereditary Fructose Intolerance 437
- Grahnén C M, Fine R N and Siles Q. Extended Hemodialysis in an Infant 271
- Mejer A and Öckerman P A. Diagnostic Problems in Glucose Galactose Malabsorption: A Case Report 714
- Lehtinen E, Gahman M and Naitan M. Protein Losses Gastroenteropathy Responding to Corticosteroid Treatment 217
- Lead H T, Trimboli J and Horvath J. A Case of Hypercalcaemia and Renal Insufficiency in Childhood Leukemia 58
- McGoldrick A E and Lomkowski P. Prolonged Survival in Neuroblastoma with Multiple Skeletal Metastases and Bone Marrow Infiltration 711
- Nilsson E. Familial Early Hypoparathyroidism Associated with Hypomagnesaemia 715
- Olaf P. Growth Hormone Response to Insulin Induced Hypoglycaemia in a Boy with Diabetes Insipidus and Short Stature before and after Treatment with Vasopressin 343
- Pollé J and Zülke J. Neoproliferative Disease in a Child with Monosomy of a C Group Chromosome 591
- Sandstrom G. Hereditary Epidermal Dysplasia: Report of Two Cases 84
- Senti G. Osteogenesis Imperfecta Congenita in One of Twins 340
- De Vries G A M and Vroom G J. Acute Leukemia with Medullary Infiltrate Simulating Perlecan at 90 Years of Age: A Case Report 415
- Vick B and Low D. Toman M, Krifsky J and Vargha J. Hyperkeratosis in Type III Cystic Fibrosis 701

## Short Communications

- Engström Y and Riebelius U. Increased Fluoride Intake by Bottle Fed Infants and its Effect 424
- Giant C A and Robertson H. Hyaline Membranes in Bronchial Artery Supplied Areas of the Human Neonatal Lung: A Microtopographical and Histological Study 705
- Zanchetta F and Tancos R. Methylmalonic Acidemia and Vitamin B<sub>12</sub> Dependency 88

## Proceedings of Paediatric Societies

- Proceedings of the Danish Paediatric Society Meeting April 9 May 28 1969 347
- Proceedings of the Danish Paediatric Society Meeting Sept 25 Oct 8 1969 348
- Proceedings of the European Society for Paediatric Gastroenterology Meeting Sept 15 1969 443
- Proceedings of the European Society for Paediatric Gastroenterology Meeting Sept 16 1969 454
- Proceedings of the European Society for Paediatric Gastroenterology Meeting Sept 18 1969 461
- Proceedings of the European Society for Paediatric Research Meeting Sept 16 1969 454
- Proceedings of the Finnish Paediatric Society Meeting May 27 May 30-31 1969 110
- Proceedings of the Finnish Paediatric Society Meeting June 11 1969 116
- Proceedings of the Finnish Paediatric Society Meeting Oct 18 1969 440
- Proceedings of the Finnish Paediatric Society Meeting Dec 13 1969 442
- Proceedings of the Norwegian Paediatric Society Meeting June 12 14 1969 226
- Proceedings of the Scandinavian Society of Paediatric Pathology Meeting June 12 14 1969 100
- Proceedings of the Scandinavian Association of Paediatric Surgeons Meeting Sept 17 19 1969 475
- Proceedings of the Scandinavian Society for Perinatal Medicine Meeting Jan 30 1970 596

## Supplements

- Sanjar Davi M, Carriote Jacques Rouleux Lydia and van Veen André. Infant Feeding and Weaning Practices in a Rural Preindustrial Setting: A Sociocultural Approach (Supplement 200)
- Sutherland Lynette L, Harbort Phyllis Bala John U, Bailey John D and Lynch Matthew J. Battered Syndrome: A Report of Four Cases Including Three in One Subject with a Comparative Histologic Evalua-

- Lowc Syndrome Observations on the Amino Acid Metabolism in a 2 Year Old Affected Boy 631
- Halikowski B Kucharska K Garwicz S Wyszowski J and Sancerz-wypach K The Results of Immunosuppressive Therapy in Chronic Renal Diseases in Children and the Assessment of Erythroblasts Behaviour 44
- Halikowski B Cyklic R Armata J Garwicz S Wyszowski J and Garapich M Cytosine Arabinoside Administered Intrathecally in Cerebromeningeal Leukemia 164
- Hall B Somatic Deviations in Newborn and Older Mongoloid Children A Follow up Investigation 199
- Halvorsen S Stokke O and Eldjarn L Abnormal Patterns of Urine and Serum Amino Acids in Methylmalonic Aciduria 28
- Hälgartner M W Lanziowski P and Smith C H The Use of Azathioprine in Refractory Idiopathic Thrombocytopenic Purpura in Children 409
- Holmberg L Solitary Mastocytoma 558
- Hoyeraal H M Larmvik J and Moe P J Congenital Hypoplastic Thrombocytopenia and Cerebral Malformations in Two Brothers 185
- Iolster N J Blood Coagulation in Children with Cystic Congenital Heart Disease 351
- Karitzky D Pringsheim W and Kunzer W Fibrinogen and Fibrinolysis in the Respiratory Distress Syndrome Observation during the First Day of Life 281
- Laron Z Karp M and Dolberg L Juvenile Hypothyroidism with Testicular Enlargement 317
- Lempert R and Lulequist B Dislocation of the Proximal Epiphysis of the Humerus in Newborns Report of Two Cases and Discussion of Diagnostic Criteria 377
- Lenard H G and Pennigstorff H Alterations in the Sleep Patterns of Infants and Young Children Following Acute Head Injuries 572
- Leysen H Kamel M Weng T R and Kruger K Expiratory Flow Rates Determined by Wedge and Water Spirometer in Children and Young Adults 648
- Lindblad B S The Venous Plasma Free Amino Acid Levels during the First Hours of Life I After Normal and Short Gestation and Gestation Complicated by Hypertension With Special Reference to the Small for Dates Syndrome 13
- Lindblad B S Rahimtoola R J and Khan N The Venous Plasma Free Amino Acid Levels during the First Hours of Life II In a Lower Socio-economic Group of a Refugee Area in Karachi West Pakistan with Special Reference to the Small for Dates Syndrome 21
- Linde L M Rasof B and Dunn O J Longitudinal Studies of Intellectual and Behavioral Development in Children with Congenital Heart Disease 169
- Lindemann R Glessing L R Merton B Loken A C and Halvorsen S Amino Acid Metabolism in Hereditary Fructosemia 141
- Lundh B Osk F A and Gardner F H Plasma Hemopexin and Haptoglobin in Hemolytic Diseases of the Newborn 121
- Lundquist A and Öckerman P A Fine Needle Aspiration Biopsy of Human Liver for Enzymatic Diagnosis of Glycogen Storage Disease and Gargoylism 293
- Meerwase G W and Lindquist B Glucose-Galactose Malabsorption Studies on the Intermediate Carbohydrate Metabolism 74
- Moe P J Hemoglobin Hematocrit and Red Blood Cell Count in Capillary (Skin Prick) Blood Compared to Venous Blood in Children 49
- Moe P J Serum Haptoglobin Level in Disseminated Malignant Diseases in Children 381
- Nilsson I M and Öckerman P A The Bleeding Disorder in Hepatomegalic Forms of Glycogen Storage Disease 127
- Olegård R and Svennerholm L Fatty Acid Composition of Plasma and Red Cell Phospholipides in Full Term Infants and their Mothers 637
- Richardson S A Patterns of Medical and Social Research in Pediatrics 265
- Savast J Nilsson L Å and Hanson L Å C React Protein in Serum from Infants as Determined by Immunodiffusion Techniques I Healthy Infants 25
- Savast J Nilsson L Å and Hanson L Å C React Protein in Serum from Infants as Determined by Immunodiffusion Techniques II Infants with Viral Infections 676
- Senterre J and Karlberg P Respiratory Quotient Metabolic Rate in Normal Full Term and Small for Date Newborn Infants 653
- Simola S Jouppila R Salmu A and Pohjonen I Encephalomeningitis in Children Associated with Adenovirus Type 7 Epidemic 310
- Sommerschield H C Grotte G and Hallin K Maximum Tubular Reabsorption of Water A Preliminary Study of Renal Concentrating Capacity in Children with Chronic Pyelonephritis 513
- Ståhlberg H Sudden Unexpected Death in Infancy Autopsy Study 685
- Ströder J and Kasal P Evaluation of Phagocytosis Rackets 288
- Stüdnitz W Cystathioninuria in Children with Neuroblastomas with and without Metastases 80
- Vapaavuori E A and Raubi N Intensive Care Small Premature Infants I Clinical Findings and Results of Treatment 353
- Vest M Signer E Wessner K and Olafsson A Double Blind Study of the Effect of Phenobarbital on Neonatal Hyperbilirubinaemia and Frequency Exchange Transfusion 681
- Väisakorpi J K Kuitunen P and Pelkonen P Intestinal Malabsorption A Clinical Study of 22 Children over 2 Years of Age 273
- Väisakorpi J K Kuitunen P and Savilahti E Frequency and Nature of Relapses in Children Suffer from the Malabsorption Syndrome with Gluten Intolerance 481
- Waalier P E Garatun Tyeksto O and Moe P Genetic Studies in Glycogen Storage Disease Type 529
- van der Zee S P M Lorumen E J P Trybels J M and Schreijen E D A M The Influence of Adenine on the Clinical Features and Purine Metabolism in Leach Nyhan Syndrome 259
- Zoppi G Shmerling D H Gaburro D and Prad A The Electrolyte and Protein Contents and Output in Duodenal Juice after Pancreozymin and Secretin

1. Scrofula in Normal Children and in Patients with Cystic Fibrosis 693  
 Ozturk I and Fluhard L B Poliovirus Antibody Status Three Years after Vaccination with Trivalent Oral Vaccine 536

## Care reports

- Achd R A Recurrent Bacteremia Following Ampicillin Treatment of Hemophilus Influenzae Meningitis 211  
 Astrakou, I and Smith S Congenital Atrial Flutter Report of a Case 587  
 Bergstrom C G and Nilsson K O Neonatal Meningitis Caused by Salmonella Thompson 427  
 Eber C P, Rosen R C, Northway W H Jr, Owe S, Smith P, Dwyer W J and Brand M M Rapid Growth and Centriobulbar Emphysema after Suctioning Tracheas and Continuous Exposure to Low Concentration of Oxygen 697  
 Ford Christensen M and Therkelsen A J A Case of the XXXXX Chromosome Anomaly with Four Maternal X Chromosomes and Diabetic Glucose Tolerance 706  
 Grant D B, Alexander F W and Sealers J W T Abnormal Tyrosine Metabolism in Hereditary Fructose Intolerance 457  
 Grubisic C M, Foye R W and Stokes Q Extended Hemodialysis in An Infant 221  
 Kasper K and Ockerman P A Diagnostic Problems in Glucose Galactose Malabsorption: A Case Report 14  
 Leibel E, Gerslitz M and Nitzan M Protein Losing Gastroenteropathy Responding to Corticosteroid Treatment 17  
 Lund H T, Tronborg J and Hornum I A Case of Hypertension and Renal Insufficiency in Childhood Sarcoidosis 587  
 McGonigal, K E and Laskowski P Prolonged Survival in Neuroblastoma with Multiple Skeletal Metastases and Bone Marrow Infiltration 711  
 Nilsson E, Finkvist E Early Hypoparathyroidism Associated with Hypomagnesemia 715  
 Oksa P Growth Hormone Response to Insulin Induced Hypoglycemia in a Boy with Diabetes Insipidus and Short Stature before and after Treatment with Vasopressin 343  
 Pollak J and Zilka J Hypoproliferative Disease in a Child with Metastases of a C Group Chromosome 191  
 Semoroff G Hereditary Ectodermal Dysplasia Report of Two Cases 94  
 Smith O Osteogenesis Imperfecta Congenita in One of Twins 330  
 De Vase, G A M and Vase, G P Acute Leukemia with Medullary Fibrosis Simulating Pericarditis 90  
 Veremart, S Silver's Syndrome 435  
 Visk B, Edvard D, Tomas M, Krifsky J and Votavsky J Hyperhidrosis in Type III Glycogen

## Review Articles

- Hagberg B and Sallanpaa M Benign Intracranial Hypertension (Pseudotumor Cerebri) Review and Report of 18 Cases 328  
 Lenard H G Sleep Studies in Infancy Facts Concepts and Significance 572  
 Oiler J and Nielsen A Nutritional Status and Inter-uterine Telangiectases Incidence in Danish School Children 416

## Short Communications

- Ericson Y and Rubelius U Increased Fluoride Intake by Bottle Fed Infants and its Effect 44  
 Grant C A and Robertson B Hyaline Membranes in Bronchial Artery Supplied Areas of the Human Neonatal Lung: A Macroscopical and Histological Study 208  
 Zappellio F and Tencones R Methylmalonic Acidemia and Vitamin B<sub>12</sub> Dependency 83

## Proceedings of Paediatric Societies

- Proceedings of the Danish Paediatric Society Meetings April 9 May 28 1969 347  
 Proceedings of the Danish Paediatric Society Meetings Sept 23 Oct 8 1969 348  
 Proceedings of the European Society for Paediatric Gastroenterology Meeting Sept 15 1969 443  
 Proceedings of the European Society for Paediatric Gastroenterology Meeting Sept 16 1969 454  
 Proceedings of the European Society for Paediatric Gastroenterology Meeting Sept 18 1969 461  
 Proceedings of the European Society for Paediatric Research Meeting Sept 16 1969 454  
 Proceedings of the Finnish Paediatric Society Meetings May 27 May 30-31 1969 110  
 Proceedings of the Finnish Paediatric Society Meeting June 11 1969 116  
 Proceedings of the Finnish Paediatric Society Meeting Oct 18 1969 440  
 Proceedings of the Finnish Paediatric Society Meeting Dec 13 1969 442  
 Proceedings of the Norwegian Paediatric Society Meeting June 12 14 1969 226  
 Proceedings of the Scandinavian Society of Paediatric Pathology Meeting June 12 14 1969 100  
 Proceedings of the Scandinavian Association of Paediatric Surgeons Meeting Sept 17 19 1969 463  
 Proceedings of the Scandinavian Society for Perinatal Medicine Meeting Jan 30 1970 96

## Supplements

- Saenger Dora M, Christos Joaquim, Rosales Lydia and van Veen André Infant Feeding and Weaning Practices in a Rural Perinatal Setting: A Sociocultural Approach (Supplement 200)  
 Sutherland Lynette E, Harrold Phyllis, Balis John U, Bailey John D and Lynch Matthew J Barter's Syndrome: A Report of Four Cases Including Three in One Subgroup with Comparative Histologic Evaluation



## RELATIONSHIP BETWEEN GLOMERULAR FILTRATION RATE AND RADIOLOGICAL APPEARANCE OF THE RENAL PARENCHYMA IN CHILDREN

U. BERG, A. APERIA, O. BROBERGER, A. EKENGREN and N. O. ERICSSON

From the Department of Pediatrics and Radiology, Crown Princess Lovisa's Children's Hospital and the Department of Paediatric Urology, Karolinska sjukhuset, Stockholm, Sweden

The present study is an attempt to reveal the functional significance of renal parenchymal changes as demonstrated by IVP's. The patients included in this study were generally referred to the radiological examination because of the history of one or more urinary tract infections. The study was necessitated by (1) the old observation of an often poor correlation between clinical history and radiological finding, (2) the inevitable question in case of significant differences in kidney size: shall nephrectomy of the small kidney be carried out and will that abolish the source of infection?

The functional evaluation has in the present report been limited to the glomerular filtration rate<sup>1</sup> as determined by the clearance of inulin. In order to fulfil the outlined intentions selective renal studies were carried out. This was accomplished by external ureteral compression. Since this method has not been used previously in childhood practice, this report will also include a methodological evaluation.

### MATERIAL

The material included 33 patients aged 3 to 15 years. All these patients except two have at one time or another had clinical signs of urinary tract infection. The patients did not have any clinical or bacteriologi-

cal signs of infection at least 2 months preceding the study.

For radiological classification the patients have been grouped on the basis of the intravenous pyelography (IVP). The radiological studies which included an IVP as well as a micturition cystogram generally preceded the functional studies by 1 to 6 months. For various reasons the main purpose of this work has been to evaluate the function of the "radiological small kidney" Group I (Table 1) therefore consists of patients where the IVP has demonstrated one kidney that has been significantly small in relation to the size of the patient. The size of the kidney has been such that the question has been raised whether the function of this kidney could be more than 20% of the total renal function. Thus all of the patients have been referred to nephrectomy. The clinical motivation for this was to abolish the source of infection. Fig. 1 including all the patients in group I demonstrates the lateral shape of the small kidney as compared with the lateral shape of a normal kidney of a subject in the corresponding age group and sex. No attempt has been made for a more precise calculation of the kidney size since the methods presently used are controversial and very rough. In only one of the patients in group I, L. N., was there any sign of more than slight parenchymal reduction on the contralateral side.

As a reference two additional groups of patients have been studied. The control group II (Table 2) consists of patients with a normal IVP. Two of these patients had no history of urinary tract infection, while the third, L. J., was considered to have had one prolonged infection with symptoms mainly of cystitis. Group III (Table 3) consists of patients where the IVP shows scarring of one or both kidneys. The parenchymal reduction was however so small that it was not considered of any importance for renal function.

In addition to the radiological classification an attempt has been made to group the patients accord-

Supported by Swedish Medical Research Council Grant B 67 19 x 7049-01A.

Abbreviations: Glomerular filtration rate = GFR.



# RELATIONSHIP BETWEEN GLOMERULAR FILTRATION RATE AND RADIOLOGICAL APPEARANCE OF THE RENAL PARENCHYMA IN CHILDREN

U. BERG, A. APERIA, O. BROBERGER, A. ELENGREN and N. O. ERICSSON

From the Department of Pediatrics and Radiology, Crown Princess Lovisa's Children's Hospital and the Department of Pediatric Urology, Karolinska Institute, Stockholm  
S. edén

The present study is an attempt to reveal the functional significance of renal parenchymal changes as demonstrated by IVP's. The patients included in this study were generally referred to the radiological examination because of the history of one or more urinary tract infections. The study was motivated by (1) the old observation of an often poor correlation between clinical history and radiological finding, (2) the inevitable question in case of significant differences in kidney size: shall nephrectomy of the small kidney be carried out and will that abolish the source of infection?

The functional evaluation has in the present report been limited to the glomerular filtration rate<sup>1</sup> as determined by the clearance of inulin. In order to fulfil the outlined intentions selective renal studies were carried out. This was accomplished by external ureteral compression. Since this method has not been used previously in childhood practice this report will also include a methodological evaluation.

## MATERIAL

The material includes 33 patients aged 3 to 15 years. All these patients except two have at one time or another had clinical signs of urinary tract infection. The patients did not have any clinical or bacteriological

signs of infection at least 2 months preceding the study.

For radiological classification the patients have been grouped on the basis of the intravenous pyelography (IVP). The radiological studies which included an IVP as well as a micturition cystogram generally preceded the functional studies by 1 to 6 months. For various reasons the main purpose of this work has been to evaluate the function of the radiologically small kidney. Group I (Table 1) therefore consists of patients where the IVP has demonstrated one kidney that has been significantly small in relation to the size of the patient. The size of the kidney has been such that the question has been raised whether the function of this kidney could be more than 20% of the total renal function. Thus all of the patients have been referred to nephrectomy. The clinical motivation for this was to abolish the source of infection. Fig. 1 including all the patients in group I demonstrate the lateral shape of the small kidney as compared with the lateral shape of a normal kidney of a subject in the corresponding age group and sex. No attempt has been made for a more precise calculation of the kidney size since the methods presently used are controversial and very rough. In only one of the patients in group I (L. N.) was there any sign of more than slight parenchymal reduction on the contralateral side.

As a reference two additional groups of patients have been studied. The control group II (Table 2) consists of patients with a normal IVP. Two of these patients had no history of urinary tract infection, while the third (L. J.) was considered to have had one prolonged infection with symptoms mainly of cystitis. Group III (Table 3) consists of patients where the IVP shows scarring of one or both kidneys. The parenchymal reduction was however so small that it was not considered of any importance for renal function.

In addition to the radiological classification an attempt has been made to group the patients accord-

Supported by Swedish Medical Research Council Grant B 67 19 x 7049-01A

Abbreviation: Glomerular filtration rate = GFR





Table 1 (cont.)

Patient and birth day	Group	Clinical history	Radiological finding		Glomerular filtration rate ml/min/1.73 m <sup>2</sup>			
			Small kidney	Contra lateral kidney	Small kidney	Contra lateral kidney	Small kidney m. of contra lateral kidney	Small kidney as % of total renal function
IVP								
K. G. female 630930	B	Bacteremia 1 yearly. Moderate symptoms. Prompt treatment.	Small with somewhat reduced contrast density. Deformation of calices.	Renal parenchyma normal in size. No scarring. 2 pelvis. Some of the calices deformed.	42	41	102	51
Micturition cystogram								
			Large reflux.	Reflux.				
IVP								
I. L. L. female 580123	B	First year of age poor weight gain. No urine sm. lysa made. Later asymptomatic till the age of 6 when bacteremia with slight symptoms once yearly.	Small kidney. Deformation of calices. no papillae.	Normalized kidney with good concentration of contrast medium. Slight "scar" in the lower part.	35	23	148	60*
Micturition cystogram								
			Large bladder. No reflux.	Moderate reflux.				
IVP								
I. A. female 580178	C	First symptomatic infection with bacteremia at the age of 2. High relapse frequency 3-5 yearly despite intensive antibiotic treatment.	Small kidney with good concentration of the contrast medium. Deformation of some of the calices.	Normalized kidney with good concentration of contrast medium. Slight scar in the lower part.	20	73	27	22
Micturition cystogram								
			Moderate reflux.	Moderate reflux.				
IVP								
E. M. L. female 630922	C	Since the age of 2 2-3 infections with bacteremia. Fever and left sided abdominal pain radiating dorsally.	Very small kidney with even contour and good concentration of contrast medium. Deformation of calices.	Normal.	24	83	29	22
Micturition cystogram								
			Moderate reflux.	Large reflux.				

Table 1 Group I Small kidney

Patient and birth day	Group	Clinical history	Radiological finding		Glomerular filtration rate ml/min/1.73 m <sup>2</sup>			
			Small kidney	Contra lateral kidney	Small kidney	Contra lateral kidney	Small kidney in of contra lateral kidney	Small kidney in of total renal function
C L male 531215	A	Clinical and bacterial evidence of only one urinary tract infection (age 12 years)	Very small but with good and even concentration of the contrast medium	IVP Normal	54	55	98	50
			Micturition cystogram No reflux	No reflux				
G B female 600706	B	Since the age of 3 years bacteriuria once yearly. Prompt treatment. Slight symptoms. No fever, no abdominal pain.	Very small kidney with thin parenchyma and deformation of the calices <sup>a</sup>	IVP Normal or slightly hypertrophied kidney	33	78	42	30
			Micturition cystogram Large reflux	Large reflux				
B S female 610320	B	Since the age of 8 months bacteriuria 1-2 yearly. Prompt treatment. Occasionally fever and right-sided abdominal pain.	Small kidney with signs of deformation in some of the calices <sup>a</sup>	IVP Normal kidney	33	79	41	29
			Micturition cystogram Large reflux with moderate dilatation	Large reflux with moderate dilatation				
E B female 640425	B	Since the age of 2 months bacteriuria 1-2 yearly. Prompt treatment. No fever, no abdominal pain.	Small kidney with good concentration of contrast medium in the thin parenchyma. Slightly deformed calices.	IVP Normal kidney	20	54	38	27
			Micturition cystogram Moderate reflux	Normal				
K M male 621115	B	Bacteriuria 1 yearly	Small kidney with good concentration of contrast medium in the renal parenchyma	IVP Normal sized with good concentration of contrast medium. No actual scarring but slight waist line.	17	25	67	40 <sup>b</sup>
			Micturition cystogram Moderate reflux	Normal				

Table 1 (cont.)

Patient and birth day	Group	Clinical history	Radiological finding		Glomerular filtration rate ml/min/1.73 m <sup>2</sup>			
			Small kidney	Contra lateral kidney	Small kidney	Contra lateral kidney	Small kidney in % of contra lateral kidney	Small kidney in % of total renal function
IVP								
K. G. female 630930	B	Bacteremia 1 year Moderate symptoms Prompt treatment	Small with somewhat reduced contrast density Deformation of calices	Renal pelvis chyma normal in size. No scarring. 2 pelvis. Some of the calices deformed	42	41	102	51
			Micturition cystogram					
			Large reflux	Reflux				
IVP								
I. L. L. female 380123	B	First year of age poor weight gain No urine analysis made Later symptoms to till the age of 6 when bacteremia with slight symptoms once yearly	Small kidney Deformation of calices no papillae	Normalized kidney with good concentration of contrast medium. Slight "scar" in the lower part	35	23	148	60*
			Micturition cystogram					
			Large bladder No reflux	Moderate reflux				
IVP								
I. A. female 480118	C	First symptoms to infection with bacteremia at the age of 2½. High relapse frequency 3-5 yearly despite extensive antibiotic treatment	Small kidney with good concentration of the contrast medium. Deformation of some of the calices	Normalized kidney with good concentration of contrast medium. Slight "scar" in the lower part	20	73	27	22
			Micturition cystogram					
			Moderate reflux	Moderate reflux				
IVP								
E. M. L. female 630922	C	Since the age of 2 3-5 infections with bacteremia fever and left sided abdominal pain radiating dorsally	Very small kidney with even contour and good concentration of contrast medium Deformation of calices	Normal	24	83	29 ^	22
			Micturition cystogram					
			Moderate reflux	Large reflux				

Table 1 (cont.)

Patient and birth day	Group	Clinical history	Radiological finding		Glomerular filtration rate ml/min/1.73 m <sup>2</sup>			
			Small kidney	Contra lateral kidney	Small kidney	Contra lateral kidney	Small kidney in of contra lateral kidney	Small kidney in of total renal function
IVP								
S. W female 631217	C	Since the age of 1 year 5-10 infections with bacteriuria yearly Fever occasionally	Small kidney Calices deformed *	Normal	24	68	36	27
Micturition cystogram								
			Large reflux	No reflux				
IVP								
L. N female 581222	C	Since at least the age of 3 years frequent infections with bacteriuria Fever occasionally	Significantly reduced parenchyma Uneven contour Deformed calices *	Slightly reduced parenchyma Uneven contour Deformed calices *	41	42	99	50
Micturition cystogram								
			Moderate reflux	No reflux				

\* Slight symptoms frequent micturitions (without polyuria) and dysuria

\* The expression deformation of calices includes irregularities in arrangement and shape of the calices the lack or flattening of the papillae

Study performed under general anaesthesia

ing to the clinical history. This grouping has been made particularly with regard to the estimated relapse frequency. For each group the following criteria have been required:

Group A: Clinical history of at most one infection with or without significant bacteriuria.

Group B: Clinical history of one to three urinary tract infections yearly. All those patients have had bacteriuria at the time of the symptoms. In none of the patients have there been recurrent episodes of high fever or high abdominal pain radiating dorsally.

Group C: Clinical history of three or more infections yearly confirmed by bacteriuria or recurrent episodes of high fever and one sided abdominal pain radiating dorsally and simultaneous bacteriuria.

Group I consists of 11 patients with one patient in group A, namely C. L. with one infection with significant bacteriuria. The rest of the clinical history of this patient is completely noncontributory. Six patients of group I are included in group B and four patients in group C. In group C patients I. A., S. W. and L. N. have had more than three infections yearly. One patient E. M. L. has had recurrent episodes of high fever and left-sided abdominal pain radiating dorsally and simultaneous bacteriuria.

In group II are included two patients without any

signs of urinary tract infection. One of those patients were admitted because of proteinuria (S. N.) one because of hypertension (M. J.) and the third patient (L. J.) has had one prolonged urinary tract infection with clear symptoms of cystitis.

Group III consists of six patients. None of those fulfil the criteria of group A. Three patients belong to group B and three to group C.

## METHODS

On the day before the study the patients received one to several enemas. On the day of the study they were given a standard breakfast meal and were allowed free water intake. Standard clearance technique was used (70). This includes a continuous infusion of 10 mU/ml inulin (Lævasar Gesellschaft) 0.001 g/min/kg b.w. after the prime dose of 0.05 g inulin/kg b.w. The continuous infusion was started at least 1 hour before the study. For urine sampling the bladder was catheterized with a double lumen polyethylene catheter enabling a continuous suction. Blood samples were obtained in the middle of each urine collection period from an indwelling needle in a peripheral vein. To ensure a high diuresis the patients were given a

constant infusion of 20% glucose. Glucose was chosen instead of mannitol since later studies which are now in progress intend to determine the  $T_m$  glucose in this material.

Unilateral clearance studies were accomplished in the following way. External ureteral compression technic was used as described by Bernstein & Hamby (1). The pressure was applied on the anterior abdominal wall pressing the ureter against the posterior brim of the pelvis inlet. The complete ureteral occlusion by this technic as well as the adequacy of urine sampling, was continuously checked by means of high amplifying fluoroscopy. For this purpose an intravenous injection of 60 Urografin (Scherweg) 0.5-1 ml/kg b.w. was given 5 minutes before compression. The duration of the ureter compression never exceeded 15 minutes.

The chemical analyses of media in serum and urine were carried out according to the method of Heyrowski (11). Before the analyses 0.09 g baker's yeast (0.5 ml of a 30% yeast suspension) was added to the samples (0.2 ml plasma) in order to eliminate the interference with glucose (3).

## EVALUATION OF METHOD

The validity of the technic is highly dependent on adequate urine sampling. Since the unilateral studies necessarily will be of short duration inadequate urine sampling would constitute a proportionately large error of method. Ureteral reflux might be one possible complication in bladder emptying. Since a rather high percentage of our patients have refluxes of varying degree (see Table 1 and 3) this complication must be excluded. The method has therefore been evaluated in three groups of patients namely (1) patients without signs of ureteral reflux (2) patients with moderate ureteral reflux but without signs of dilatation of ureter and/or renal pelvis and finally (3) patients with large ureteral reflux with signs of dilatation of ureter and/or renal pelvis. Fig. 2 (a and b) demonstrates typical micturition cystograms from two patients with moderate and severe reflux respectively.

Tables 4 to 6 give the GFR in the three groups. Some of the patients in these tables are not included in the later tables namely those with unilateral or bilateral hydronephrosis. These patients are excluded in the further report because hydronephrosis might influence

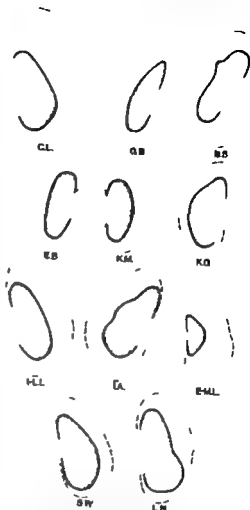


Fig. 1 The lateral shape of the small kidney of all the patients in group I (thick line). The dashed line represents the size of a normal kidney from a healthy patient of the same age.

renal function. However for methodological evaluation hydronephrosis is of no significance. In each patient in the tables the average values from two unilateral clearance determinations are given. These values have then been added (i.e. the calculated bilateral glomerular filtration rate) and compared to the results from the bladder sampling before ureteral compression i.e. the found bilateral values. The found bilateral values represent the mean of two to four clearance periods. The individual scatter between those periods does not exceed 20%. The tables demonstrate the correlation coeffi-

Table 2 Group II Normal kidney

Patient and birth day	Group	Clinical history	Radiological finding		Glomerular filtration rate ml/min/1.73 m <sup>2</sup>			
			Small kidney	Contra lateral kidney	Small kidney	Contra lateral kidney	Small kidney in of contra lateral kidney	Small kidney in of total renal function
M J male 620112	A	Hypertension of nonrenal origin (hyperaldosteronism) known since 66	Normal	IVP Normal	50	52	96	
			Normal	Micturition cystogram Normal				
S N female 560925	A	Proteinuria known since 63 No signs of nephrosis	Normal	IVP Normal	48	49	96	
			Normal	Micturition cystogram Normal				
L J female 590710	A	One infection with bacteriuria 64 Slight symptoms *	Normal	IVP Normal	72	62	116	
			Normal	Micturition cystogram Normal				

Table 3 Group III Scarred kidney

Patient and birth day	Group	Clinical history	Radiological finding		Glomerular filtration rate ml/min/1.73 m <sup>2</sup>			
			Small kidney	Contra lateral kidney	Small kidney	Contra lateral kidney	Small kidney in of contra lateral kidney	Small kidney in of total renal function
I M K female 550420	B	Since the age of 1 year in fections with bacteriuria 2-3 yearly Fever occasionally	Moderately reduced parenchyma Deformation of calices *	IVP Slightly reduced scarred parenchyma Some of the calices deformed	35	80	44	30
			No reflux	Micturition cystogram No reflux				
C N female 640720	B	Since the age of 1 year in fections with bacteriuria 1-2 yearly Slight symptoms	Slight parenchymal reduction in the lower part Calices normal	IVP Normal	57	51	107	52
			Large reflux	Micturition cystogram No reflux				

Table 3 (cont.)

					Glomerular filtration rate ml/min/1.73 m <sup>2</sup>			
Patient and birth day	Group	Clinical history	Radiological findings		Small kidney	Contra lateral kidney	Small kidney as % of contra lateral kidney	Small kidney as % of total renal function
			Small kidney	Contra lateral kidney				
IVP								
J J male 580531	B	61 64 1 3 infections yearly with bacteriuria (fever and abdominal pain occur seasonally since 64 no clinical or bacteriological signs of infection)	Scarred kidney with moderately reduced parenchyma. Small caliculi calcions in upper part. One calic (in upper part) deformed.	Normalized parenchyma. Slight caliculi calcions in upper part.	22	47	46	32
Micturition cystogram								
			Moderate reflux	Slight-moderate reflux				
IVP								
A B female 560679	C	Since the age of 5 years 2-4 infections yearly generally with fever and abdominal pain radiating dorsally	Moderate parenchymal reduction. Very uneven contour. Several small caliculi calcions.	Normal	23	60	38	28
Micturition cystogram								
			Moderate reflux	No reflux				
IVP								
C A female 570310	C	Since the age of 4 years 4-5 infections with bacteriuria yearly. Fever occurs seasonally	Slight parenchymal reduction in lower part. Caliculi Normal.	Normal	46	43	109	52
Micturition cystogram								
			Slight reflux	No reflux				
IVP								
K C female 571028	C	Since the age of 4 years 2-3 infections yearly with bacteriuria. Symptoms generally severe with acute stress fever abdominal pain	Slight scarring. Vague actual parenchymal reduction.	Normal	49	70	70	41
Micturition cystogram								
			Large reflux	No reflux				

Slight symptoms include frequent micturitions (without polyuria) and dysuria. The expression deformation of calices includes irregularities in arrangement and shape of the calices, the lack or flattening of the papillae.



Table 2 Group II - Normal kidney

Patient and birth day	Group	Clinical history	Radiological finding		Glomerular filtration rate ml/min/1.73 m <sup>2</sup>			
			Small kidney	Contra lateral kidney	Small kidney	Contra lateral kidney	Small kidney in % of contra lateral kidney	Small kidney in % of total renal function
M J male 620112	A	Hypertension of nonrenal origin (hyperaldosteronism) known since 66	Normal	IVP Normal	50	52	96	
			Normal	Micturition cystogram Normal				
S N female 560925	A	Proteinuria known since 65 No signs of nephrosis	Normal	IVP Normal	48	49	96	
			Normal	Micturition cystogram Normal				
L J female 590710	A	One infection with bacteriuria 64 Slight symptoms	Normal	IVP Normal	72	62	116	
			Normal	Micturition cystogram Normal				

Table 3 Group III - Scarred kidney

Patient and birth day	Group	Clinical history	Radiological finding		Glomerular filtration rate ml/min/1.73 m <sup>2</sup>			
			Small kidney	Contra lateral kidney	Small kidney	Contra lateral kidney	Small kidney in % of contra lateral kidney	Small kidney in % of total renal function
I M K female 550420	B	Since the age of 1 year in fections with bacteriuria 2-3 yearly Fever occasionally	Moderately reduced parenchyma Deformation of calices <sup>a</sup>	IVP Slightly reduced scarred parenchyma Some of the tho calices deformed <sup>b</sup>	35	80	44	30
			No reflux	Micturition cystogram No reflux				
C N female 640720	B	Since the age of 1 year in fections with bacteriuria 1-2 yearly Slight symptoms	Slight parenchymal reduction in the lower part Calices normal	IVP Normal	37	53	107	52
			Large reflux	Micturition cystogram No reflux				

Table 6 The calculated and found GFR in patients with large reflux

GFR (ml/min/1.73 m<sup>2</sup> b.s.)

Patient	Right kidney GFR	Left kidney GFR	The calculated total GFR	The found total GFR
GB	33	78	111	106
HE	96	96	192	183
EA	76	46	122	122
EML	83	24	107	93
BS	33	79	112	111
SV	68	24	92	85
KO	70	49	119	121
CN	33	57	110	106
AO	42	41	83	84

0.986

$r=153$

$p<0.01$

discrepancy between paired samples is also somewhat larger. Group 3 Table 6 consists of patients with large ureteral reflux and secondary dilatation of the ureter and/or renal pelvis. It is remarkable that the correlation coefficient between estimated and experimentally found bilateral GFR is as high as 0.986. In addition the difference between paired samples is lower than 5 in 7 of 9 patients. Thus ureteral reflux of varying degree seems little to influence the adequacy of urine sampling.

The method with external ureteral compression technique is apparently of special value in a more detailed study of unilateral renal function in childhood. The other alternative of making selective renal clearance studies, namely a method involving ureteral catheterization, will be less valid in childhood practice. The two main reasons for this are (1) the size of the pediatric cystoscope will not allow the passage of sufficiently large catheters supplied with appropriate cuffs, (2) even if a sufficiently large catheter could be introduced in some of the patients a right ureteral fit around the catheter can only be expected in patients without ureteral reflux. The extremely good validity of the external ureteral compression technique has been demonstrated previously by Bernstein & Hamby (1910). Those results have been

confirmed in the present work. Furthermore this study also demonstrates the complete validity of this method even in cases with moderate or severe reflux (correlation coefficient of 0.958 and 0.986). This finding is of special value not only for the present study but also for the evaluation of the total clearance technique in children with varying degrees of refluxes. It should however be noted that the evaluation of this method has been made under the condition of rather heavy osmotic diuresis. It is still possible that studies under hydropenia can be combined with rather high methodological error.

## RESULTS

### GFR in group I

All the GFR values are expressed in ml/min/1.73 m<sup>2</sup> body surface. In only 5 out of 11 patients is the GFR of the small kidney lower than 25 ml/min, i.e. the estimated 50% reduction from the normal value for adults (20). The lowest recorded GFR was 17 ml/min. It is, however, to be noticed that this study was performed under general anesthesia. In 5 of the 11 patients the GFR of the small kidney is between 30 and 42 ml/min/1.73 m<sup>2</sup>. The function of this kidney is probably good enough for the patient to escape renal insufficiency. In patient C.L. the GFR is normal.

In the next to last column Table I the GFR of the small kidney has been given in % of the GFR of the contralateral kidney. In 4 of 11 patients the GFR of the small kidney exceeds 98% of the contralateral kidney and in none of the patients is the GFR of the small kidney lower than 27% of the contralateral kidney. Thus there appears to be little correlation between the IVP finding and the functional finding.

If on the other hand renal function is correlated not to radiological size but rather to the clinical history with number and severity of urinary tract infections, i.e. the grouping A to C, a certain correlation appears to exist. Thus the highest discrepancy in GFR between the

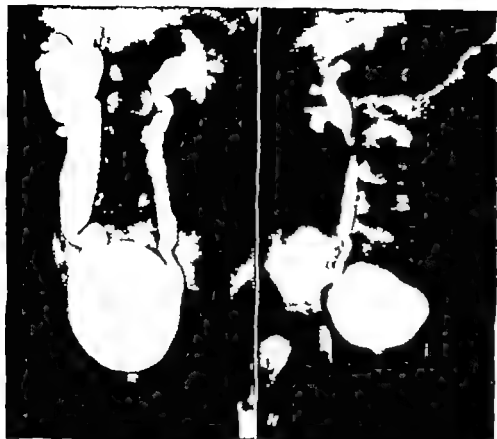


Fig 2 (a and b) Micturition cystogram from patient H S group I Table I and from patient E H group I Table I

cient and its  $t$  value. In group 1 Table 4 namely the patients without radiological signs of ureteral reflux the correlation coefficient is 0.992. It should also be noticed that the difference between paired samples rarely exceeds 5. In group 2 Table 5 namely the patients

Table 4 The calculated and found GFR in patients without signs of ureteral reflux

GFR (ml/min/1.73 m<sup>2</sup> b s)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat GFR	The found bilat GFR
LE	14	58	72	77
CG	49	39	88	88
JH	22	22	44	40
MS	77	56	133	128
AK	40	7	47	49
SEK	74	22	96	102
FP	66	88	152	151
SN	48	49	97	100
MJ	50	52	102	94
IMK	35	80	115	118

$r=0.992$   
 $t=22.2$   
 $p<0.01$

Study performed under general anesthesia

where moderate ureter reflux has been demonstrated on the micturition cystogram the correlation coefficient is still highly significant but somewhat lower namely 0.958. In addition the

Table 5 The calculated and found GFR in patients with moderate reflux

GFR (ml/min/1.73 m<sup>2</sup> b s)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat GFR	The found bilat GFR
AKB	60	39	99	97
CE	75	49	124	109
WF	42	34	76	90
CK	43	46	89	83
ILL	23	35	58	42
KM	25	17	42	40
AB	60	23	83	81
ACB	13	20	33	29
ILG	41	83	126	139
IA	20	73	93	91
EB	20	54	74	67
LN	42	41	83	80
JJ	22	47	69	69

$r=0.958$   
 $t=11.0$   
 $p<0.01$

Study performed under general anesthesia

Table 6 The calculated and found GFR in patients with large reflux

GFR (ml/min/1.73 m<sup>2</sup> b.s.)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat. GFR	The found bilat. GFR
GB	33	78	111	106
HE	96	96	192	183
EK	76	46	122	122
EML	83	24	107	93
ES	33	79	112	111
SW	68	4	9	89
KG	70	49	119	121
CN	53	57	110	106
KG	42	41	83	84

$r = 0.986$

$t = 15.3$

$p < 0.01$

discrepancy between paired samples is also somewhat larger. Group 3 Table 6 consists of patients with large ureteral reflux and second or third dilatation of the ureter and/or renal pelvis. It is remarkable that the correlation coefficient between estimated and experimentally found bilateral GFR is as high as 0.986. In addition the difference between paired samples is lower than 5 in 7 of 9 patients. Thus ureteral reflux of varying degree seems little to influence the adequacy of urine sampling.

The method with external ureteral compression technique is apparently of special value in a more detailed study of unilateral renal function in childhood. The other alternative of making selective renal clearance studies namely a method involving ureteral catheterization will be less valid in childhood practice. The two main reasons for this are (1) the size of the pediatric cystoscope will not allow the passage of sufficiently large catheters supplied with appropriate cuffs. (2) even if a sufficiently large catheter could be introduced in some of the patients a right ureteral fit around the catheter can only be expected in patients without ureteral reflux. The extremely good validity of the external ureteral compression technique has been demonstrated previously by Bernstein & Hamby (1910). Those results have been

confirmed in the present work. Furthermore this study also demonstrates the complete validity of this method even in cases with moderate or severe reflux (correlation coefficient of 0.958 and 0.986). This finding is of special value not only for the present study but also for the evaluation of the total clearance technique in children with varying degrees of refluxes. It should however be noted that the evaluation of this method has been made under the condition of rather heavy osmotic diuresis. It is still possible that studies under hydropenia can be combined with rather high methodological error.

## RESULTS

### GFR in group I

All the GFR values are expressed in ml/min/1.73 m body surface. In only 5 out of 11 patients is the GFR of the small kidney lower than 25 ml/min i.e. the estimated 50% reduction from the normal value for adults (20). The lowest recorded GFR was 17 ml/min. It is however to be noticed that this study was performed under general anesthesia. In 5 of the 11 patients the GFR of the small kidney is between 30 and 42 ml/min/1.73 m. The function of this kidney is probably good enough for the patient to escape renal insufficiency. In patient C.L. the GFR is normal.

In the next to last column Table I the GFR of the small kidney has been given in % of the GFR of the contralateral kidney. In 4 of 11 patients the GFR of the small kidney exceeds 98% of the contralateral kidney and in none of the patients is the GFR of the small kidney lower than 27% of the contralateral kidney. Thus there appears to be little correlation between the IVP finding and the functional finding.

If on the other hand renal function is correlated not to radiological size but rather to the clinical history with number and severity of urinary tract infections i.e. the grouping A, B, C a certain correlation appears to exist. Thus the highest discrepancy in GFR between the

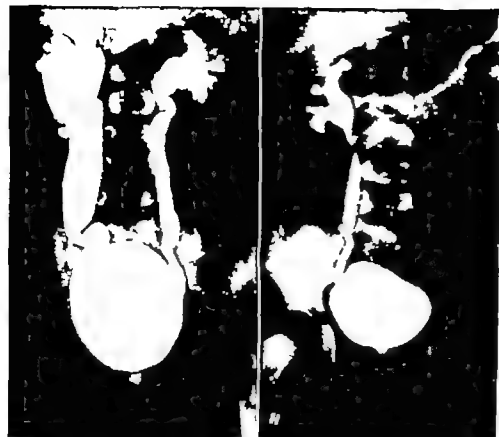


Fig 2 (a and b) Micturition cystogram from patient B S group I Table I and from patient E B group I Table I

cient and its  $t$  value. In group 1 Table 4 namely the patients without radiological signs of ureteral reflux the correlation coefficient is 0.992. It should also be noticed that the difference between paired samples rarely exceeds 5%. In group 2 Table 5 namely the patients

where moderate ureter reflux has been demonstrated on the micturition cystogram the correlation coefficient is still highly significant but somewhat lower namely 0.958. In addition the

Table 4 The calculated and found GFR in patients without signs of ureteral reflux

GFR (ml/min/1.73 m<sup>2</sup> b.s.)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat GFR	The found bilat GFR
LE	14	58	72	77
CG	49	39	88	88
JH	22	22	44	40*
MS	77	56	133	128
AK	40	7	47	49*
SEK	74	22	96	102
FP	66	86	152	151
SN	48	49	97	100
MJ	50	52	102	94
IMK	35	80	115	118

$r=0.992$

$t=22.2$

$p<0.01$

\* Study performed under general anesthesia

Table 5 The calculated and found GFR in patients with moderate reflux

GFR (ml/min/1.73 m<sup>2</sup> b.s.)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat GFR	The found bilat GFR
AKB	60	39	99	97
CE	75	49	124	109
WF	42	34	76	90
CK	43	46	89	95
ILL	23	35	58	42*
KM	25	17	42	40
AB	60	23	83	84
ACB	13	20	33	29
ILG	41	85	126	139
IA	20	73	93	91
EB	20	54	74	67
LN	42	41	83	80
MI	22	47	69	69

$r=0.958$

$t=11.0$

$p<0.01$

Study performed under general anesthesia

of renal parenchyma could vary. At the present time two main conditions predisposing to renal parenchymal reduction in children (without the clinical history of glomerulonephritis) are recognized namely (1) recurrent pyelonephritis with consequent scarring (12 17 18 22) and (2) primary renal dysplasia (2 7 8 14 15 16). Since renal dysplasia predisposes to infection the pathology of some of the kidneys studied might be a mixture of both conditions. The determinations of GFR only does not permit any further differential diagnosis between the two conditions. On the basis of the apparently good inverse correlation between the glomerular filtration rate and the relapse frequency it might however be suggested that pyelonephritic scarring would relatively more damage the nephron than would renal dysplasia.

One of the most surprising findings of this study was the generally relatively good and in some cases apparently normal function of some of the small kidneys. From what has been described by Templeton *et al* (23) it is rather likely that the dimension of the main renal artery of those small kidneys is reduced at least to some extent. It is therefore unlikely that total blood flow and vascular resistance would be exactly the same as in a normal kidney of a much larger size. It has however been suggested by other investigators (3) that during normal conditions only part of the nephron population is functioning and the rest constitutes a reserve capacity. It might therefore be suggested that in the case of the small kidney a much larger proportion of the nephron population is functioning with consequent reduction of the reserve capacity.

The other extreme in the discrepancy between radiological and functional finding, namely a relatively large kidney with proportionally low GFR, is just as noteworthy from the clinical point of view. This finding was most pronounced in patients J J and A H in group III Table 3 and K G in group I Table 1 (contralateral kidney). Patients L N (contralateral side) in group I Table 1 and C K (contralateral side) in group III Table 3 have

20% reduction in function while the parenchymal reduction judged from the IVP could be estimated to at most 5%. Since the total function in all the patients well exceeded 50% of normal the reduction in GFR could not be expected to be reflected in the usual screening tests i.e. serum creatinine and urea. The reason for the disproportionately large reduction in GFR in patients J J A H K G L N and C K cannot be defined on the basis of the present results but deserve more detailed renal function studies.

### SUMMARY

Selective renal function studies with determination of the glomerular filtration rate (minum clearance) have been carried out in more than 30 children aged 3 to 15 years. Most of the patients had a history of one or more urinary tract infections as well as pathological IVP findings of varying degree.

The selective studies were accomplished by means of external ureteral compression. The method proved to have an extremely good validity even in cases of severe ureteral reflux with dilatation of ureter and pelvis.

No correlation could be found between radiological size of the kidney and glomerular filtration rate. In almost half of the patients with significant unilateral reduction of the renal parenchyma the glomerular filtration rate of this side was found to be more than 40% of the total glomerular filtration rate. In contrast the glomerular filtration rate of several of the kidneys estimated to be of normal size was significantly reduced. It is obvious from these findings that nephrectomy must never be carried out until selective renal function studies have been made.

An additional finding was an apparently good correlation between the clinical and functional finding i.e. the glomerular filtration rate appeared to be inversely correlated to the relapse frequency of the urinary tract infections. The fact that the functional finding correlated well with the clinical finding but not with the

two kidneys is found in group C, i.e. the patients with the most severe and frequent relapses. Also in group B i.e. the patients with moderate severe infections, there is a certain but less pronounced discrepancy in GFR between the two kidneys. On the other hand the patient in group A who presented with the history of only one urinary tract infection was found to have a normal GFR on both sides, thus also of the small kidney.

### *GFR in group II*

Group II consists of patients with normal IVP's and with one exception without any history of urinary tract infection. The average GFR of one kidney is 55 ml/min/1.73 m. This value corresponds rather well to the reported normal values for the adults (20).

### *GFR in group III*

Group III consists of patients with scarred kidneys. Naturally the scarring was not completely symmetrical. Thus one kidney was always somewhat more scarred than the other one. The result of this kidney is presented in the "small kidney" column. In 3 of the 6 patients the GFR of both kidneys exceeded 40 ml/min/1.73 m. In the next to last column the function of the two kidneys is compared. In 4 of the 6 patients the GFR of the most scarred kidney is significantly lower than the GFR of the contralateral side. In 2 of the patients the GFR is approximately the same in the two kidneys. Thus again no correlation can be demonstrated between radiological and functional finding. In contrast to group I the correlation between clinical finding and functional finding also seems poor among those patients. Thus 2 of 3 of the patients in group C, i.e. patients with high relapse frequency have good function on both sides (C K and K G).

## DISCUSSION

The immediate implication of this study is the absolute necessity of selective renal function

determinations before nephrectomy is carried out. The two main indications for nephrectomy in children with recurrent urinary tract infections and unilateral reduction of the renal parenchyma are (1) renal hypertension and (2) chronic pyelonephritis more or less resistant to antibiotic treatment. It is obvious from group I Table 1 that in most of those patients nephrectomy would be contraindicated.

The validity of these indications for unilateral nephrectomy can be seriously questioned. Renal hypertension is seldom due to outflow of hypertensive substances from one single kidney. In most cases selective renal studies will show that both kidneys are damaged with regard to renal circulation. Chronic pyelonephritis cannot be regarded as a more valid indication for nephrectomy. It would indeed be optimistic to expect a chronic urinary tract infection of long standing to leave one kidney intact. In addition there is at present great controversy on what is the primary source of infection. A defect in the defence against ascending infections i.e. a defective resistance of the urinary bladder may be of primary importance (4, 5, 19, 26).

Thus there is right now no clearcut indication for nephrectomy in pediatric patients with nephropathies other than tumors. On the contrary the present work has demonstrated that in most cases nephrectomy is contraindicated. In none of the patients presented was the renal function of the small kidney less than 20% of the total renal function and in 6 of the patients the function was more than 40% of the total renal function. Another factor contributing to the contraindications towards nephrectomy is the commonly found functional reduction of the contralateral kidney. It is hardly likely that a kidney with an already reduced renal function would be able to hypertrophy and increase its function beyond what is normal for one kidney.

The lack of correlation between radiological and functional finding deserves a few comments. One likely reason for this is the fact that the pathological basis for the reduction

## THE VENOUS PLASMA FREE AMINO ACID LEVELS DURING THE FIRST HOURS OF LIFE

*I. After Normal and Short Gestation and Gestation Complicated by  
Hypertension With Special Reference to the Small for Dates Syndrome*

B S LINDBLAD

*From the Departments of Paediatrics at Crown Princess Lovisa's Children's Hospital  
and the Department of Chemistry II Karolinska Institute, Stockholm, Sweden*

Many babies who at birth are unduly small for the period of gestation (small for dates - SFD) show certain clinical features indicating impairment of prenatal nutrition (36).

The homeostasis of plasma free amino acids changes characteristically in experimental protein undernutrition (low intake of dietary nitrogen) (1, 2, 28, 31). This change is also seen in the clinical syndrome of protein deficiency (3, 11, 16, 27, 33, 35).

The aim of the present study is to investigate whether this characteristic change in the homeostasis of plasma free amino acids is also valid for the SFD newborn. The SFD syndrome has a multifactorial background (5). This study deals with SFD babies of mothers with hypertensive disorder of pregnancy. This maternal condition has been shown to be associated with low birth weight and short length for gestational age of the babies in an earlier investigation (20).

### MATERIAL

The material is based on a study of 47 newborns (18 normals, 9 early borns and 20 newborns of mothers with hypertension during pregnancy). Essential

This study was made possible through grants from AB Sjöperss Fund for Nutritional Research and grant no. 2583 from the Swedish Medical Research Council.

neutral amino acid levels of plasma were determined in all cases at different times during the first hours of life. In 25 of the cases (9 normals, 8 early borns and 8 newborns of mothers with hypertension during pregnancy) one complete determination of 18 free amino acid levels was made.

The mothers were all without history of previous disease. The normal and short gestation group had a blood pressure below 140/90 and no protein or glucose in their urine during the actual pregnancy. All of them were Rh positive and no child showed hyperbilirubinaemia during the experimental period. The normals and early borns had a crown vaginal delivery while 6 of the hypertensive mothers were delivered by caesarean section because of low urinary oestriol excretion in 5 cases and intrauterine asphyxia in one case. The cord was wound 3 times around the neck of the cephalic child and as cord obstruction is known to lower the amino acid levels of cord plasma (8) this case is separately presented in Fig. 1 and was excluded from the survey. The criteria of hypertensive disorder of pregnancy have been given in an earlier work (20). There was no correlation between time of onset of hypertension and birth weight reduction. The mothers were treated from the time of first noted hypertension with Apresolin® and Reser® (Polythazide) with extra peroral potassium. All three of the newborns of the hypertensive group whose mothers had not been treated during pregnancy had a birth weight or length below -2 s.d. according to the Swedish standard for gestational age and sex (12). Oestriol excretion below 10 mg/day was noted during the last week of pregnancy in 5 hypertensive cases, out of whom one had a birth weight or length below -2 s.d.

The Apgar score was normal at 4 min of age and through the experimental period. The normals and early borns had a birth weight and length within  $\pm 1$  s.d. for gestational age and sex. The newborns of



radiological size of the kidney suggests various etiologies for the parenchymal reduction

### ACKNOWLEDGEMENT

The authors are indebted to Mrs Karin Egeld and Mrs Guye Svensson for excellent technical assistance

### REFERENCES

- Bernstein L M & Hamby W M Unilateral urine sampling utilizing external ureteral compression *New Eng J Med* 268 1093 1963
- Bernstein J Developmental abnormalities of the renal parenchyma—renal hypoplasia and dysplasia *Path Ann* 3 213 1969
- Coelho J B & Bridley S E Function of the nephron population during haemorrhagic hypotension in the dog with special reference to the effects of osmotic diuretics *J Clin Invest* 43 386 1964
- Cox C E & Hinman P Jr Experiments with induced bacterium vesical emptying and bacterial growth on the mechanism of bladder defence to infection *J Urol* 86 739 1961
- Cox C E Lacy S S & Hinman P Jr Urethra and its relationship to urinary tract infection II Urethral flora of female with recurrent urinary infection *J Urol* 99 632 1968
- Emmett J L Alvarez Ieremi J J & McDonald J R Atrophic pyelonephritis versus congenital renal hypoplasia *JAMA* 149 1970 1952
- Ericsson N O & Iversen B I Renal dysplasia and pyelonephritis in infants and children Part I *Arch Path* 66 255 1958
- Ericsson N O & Iversen B I Renal dysplasia and pyelonephritis in infants and children Part II *Arch Path* 66 264 1958
- Hamby W M & Bernstein L M Measurement of unilateral renal function by external arterial compression *Circ* 31 679 1965
- Hamby W M Blumberg M D Simpson K & Bernstein L M Unilateral renal function measurement external ureteral compression compared with ureteral catheterization *J Lab Clin Med* 71 722 1968
- Heyrowsky A A new method for the determination of insulin in plasma and urine *Clin Chim Acta* 1 470 1956
- Hodson C J The radiological contribution to ward the diagnosis of chronic pyelonephritis *Radiology* 88 857 1967
- Ladefoged J & Pedersen P Relationship between roentgenological size of the kidney and the kidney function *J Urol* 99 239 1968
- Marshall A G The persistence of foetal structure in pyelonephritic kidneys *Brit J Surg* 41 38 1953
- Scars of the renal cortex *J Path Bact* 71 95 1956
- Scars of the renal cortex *J Path Bact* 91 225 1969
- Michie A J & Michie C R Kidney function in unilateral pyelonephritis I Clinical data *Amer J Med* 22 179 1957
- Michie A J Michie C R & Rattu M C Kidney function in unilateral pyelonephritis II Physiologic interpretations *Amer J Med* 23 190 1957
- Norden C W Green G M & Kass E H Antibacterial mechanisms of the urinary bladder *J Clin Invest* 47 2659 1969
- Smith H W *The kidney. Structure and function in health and disease* Oxford University Press, New York 1951
- Stanley T A Fair W R Timothy M M & Chung H K Antibacterial nature of prostatic fluid *Nature* 218 444 1969
- Struss M B & Welt L G *Diseases of the kidney* Little Brown and Comp Boston 1963 p 469
- Templeton A W & Thompson I M Aortic graphic differentiation of congenital and acquired small kidneys *Arch Surg* 97 114 1968
- Wunberg J Renal concentration capacity during acute nonobstructive urinary tract infections in infancy and early childhood *Acta Paediatr Scand* 47 635 1958
- Renal function studies in infants and children with acute nonobstructive urinary tract infections *Acta Paediatr Scand* 48 577 1959
- Vivaldi E Cotran R Zantwill D P & Kass E H Ascending infection as a mechanism in the pathogenesis of experimental non obstructive pyelonephritis *Proc Soc Exp Biol Med* 107 247 1959

Submitted July 3 1969

Accepted July 28 1969

(U B) Dept of Pediatrics  
Kronprinsessan Lovisas Barnsjukhus  
Polhemsgatan 30  
112 30 Stockholm  
Sweden

Key words Childhood pyelonephritis renal parenchymal reduction renal dysplasia split function test



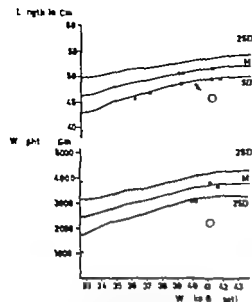


Fig. 1 Weight and length at birth in relation to the length of the gestational period of the cases where pregnancy was complicated by hypertension. The filled circles represent the group characterized as the growth retarded group. The crosses represent those growth retarded newborns who developed neonatal hypoglycaemia. The encircled filled circles represent the case with intrauterine asphyxia. For the sake of simplicity both sexes have been plotted onto the Swedish standard for boys (12).

mothers with hypertension had weight and length below  $-1$  SD in 12/20 cases. These babies are referred to as the growth retarded group (Fig. 1). Weight or length below  $-2$  SD was present in 10/20 cases and weight and length below  $-2$  SD in 6/20 cases. The normals had a gestational age of 38–41 weeks, the early born less than 36 weeks and the hypertensive group 36–41 weeks. The babies showed no malformations or infections during the neonatal period.

## EXPERIMENTAL

The babies were all transferred to an incubator in a unit for newborns within one hour after delivery. The stomach was emptied by tubing and sucking immediately after birth.

1–2 ml of blood was drawn from the cubital vein into a heparinized tube at different times during the first hours of life. The first feed was given at 6 hours of age. In 12 cases where the first feed (10 g of pooled breastmilk) was given at 4 hours of age a sample of 3–4 ml of blood was drawn before and one hour after the feed. In these cases blood for glucose estimation was collected by heel puncture before the first feed. After this the full term babies were taken out of the incubator. Subsequent samples were drawn 3–4 hours after the latest feed (immediately before the next feed). In 6 normals the first period of fasting

was prolonged and a blood sample of 3–4 ml was drawn before and one hour after the first feed (30 g of pooled breastmilk) at 44–51 hours of age. These babies had received 10–20 ml of 10% glucose solution 6 times daily during this period. Urine was collected in a sterile plastic urinal bag at 4–7 hours of age from 16 cases (all boys because of the greater possibility of preventing contamination). These urine samples were frozen immediately after micturition.

## ANALYTICAL METHODS

Collection of samples, storage and deproteinization has been described earlier (19). The levels of 18 free amino acids and urea were determined in the 4 and 48 hour samples of 0.5–1.0 ml plasma with a Beckman 120 B automatic amino acid analyzer (19).

The neutral essential amino acid levels were determined in 0.3 ml of plasma according to the ion exchange chromatography method described in an earlier report (21).

A further modification allowed reproducible determinations of proline, glycine, alanine and valine levels of the urine samples. The pH of the urine was brought to 2.0 by addition of 6 N HCl. Without further preparation 0.2–0.5 ml were put directly onto the column. The pH of the elution buffer was lowered from 3.43 to  $3.06 \pm 0.03$  by addition of concentrated HCl. The temperature was 60°C. The two fold linear amplification of the recorder was omitted (Fig. 2).

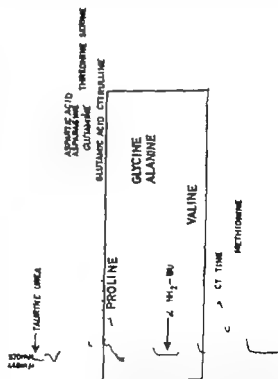


Fig. 2 Modified standard run of ion-exchange chromatography for determination of urinary concentrations of proline, glycine, alanine and valine. 50  $\mu$ mol of each amino acid was loaded on the column.

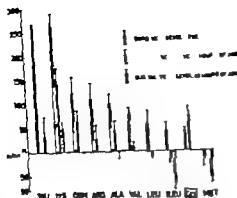


Fig 3 Change of the free amino acid levels of plasma during the first hours of life of the fasting normal newborn. The 4 hour level is indicated if different from the cord vein level (19) the 48 hour level if different from the 4 hour level. The other 8 amino acid levels investigated showed a not significant decrease and were a not significant increase during this period. The normal infant level is that of infants 9 months to 2 years of age (29).

$\beta$  hydroxybutyrate was determined according to Persson (24). Plasma albumin was determined by electrophoresis on cellulose acetate (30). Blood glucose was estimated by the enzymatic method of Marks (22).

## RESULTS

**Normal newborns.** At 1-3 hours of age the venous plasma levels of the branch-chained amino acids (valine, leucine, isoleucine) and

Table 1 Normal newborns

Cord vein plasma levels of free amino acids (CB(19)) are compared to those of the cubital vein plasma at 4 hours of age (CUB). Mean and s.d. are given in  $\mu\text{mol/l}$  plasma.

Amino acid		Mean	s.d.	Significance of difference
Valine	CB	10	224	33
	CUB	12	139	20
Leucine	CB	10	118	32
	CUB	12	67	13
Isoleucine	CB	10	6	12
	CUB	12	36	5
Lysine	CB	10	318	32
	CUB	5	212	11
Alanine	CB	10	441	75
	CUB	9	311	63
Glycine	CB	10	239	33
	CUB	9	293	48

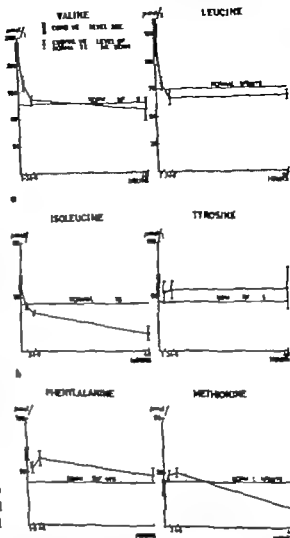


Fig 4 Mean  $\pm$  2 s.d. of the plasma levels of neutral essential amino acids of the normal newborn during the first hours of life. The horizontal line indicates the normal fasting levels of infants 9 months to 2 years of age (29).

phenylalanine showed a decrease from cord levels (Fig 4).

At 4 hours of age the branch-chained amino acid levels showed a further decrease. The levels of lysine and alanine were considerably lower than their cord levels while the glycine level was increased. The ornithine/arginine quotient seems to be increased compared to cord value (Fig 3). Urea was not significantly increased (Fig 6 a). See Table 1.

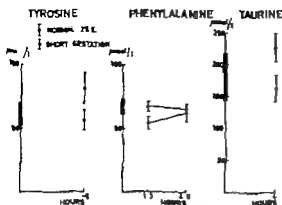


Fig 5 Change of the free amino acid levels of plasma of the early born during the first hours of life. Mean  $\pm$  s.e. is indicated.

When fasting was prolonged until 48 hours of age isoleucine, lysine and alanine levels showed a further decrease. The taurine and methionine levels were low compared to cord levels (Fig 3).

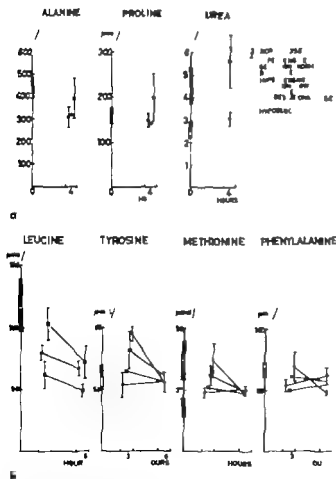


Fig 6 Differences from the normal free amino acid levels of plasma in the newborns of mothers with hypertensive disorder.

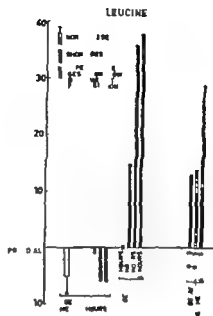


Fig 7 Plasma leucine level one hour after the first feed of 10 g of pooled breastmilk during the first day of life and 30 g after prolonged neonatal starvation.

One hour postprandial levels after 10 g of breastmilk at 4 hours of age showed no significant or consistent differences from preprandial levels. Thirty grams of breastmilk after 48 hours of fasting gave an increase of leucine, isoleucine and a decrease of glycine and the glycine/valine quotient of plasma one hour postprandially (Fig 7).

**Newborns with short gestational period.** At 1-3 hours of age there were higher levels than in normals of phenylalanine and after 4-6 hours of fasting higher levels than in normals of taurine and tyrosine (Fig 5). One hour after 10 g of breastmilk, given at 4 hours of age there seemed to be a consistent non significant increase of alanine only.

**Newborns of mothers with hypertensive disorder.** Differences from the normal and short gestational group.

After 1-3 and 4-6 hours of neonatal fasting the branch-chained amino acids showed lower levels if pregnancy had been complicated by hypertension. In the case of low birth weight for gestational age however the levels were increased at 1-3 hours and normal at 4-6 hours. There seems to be a more rapid decrease of the

Table 2 Newborns of mothers with hypertensive disorder and normal weight at birth according to gestational age (A) and newborns of mothers with hypertensive disorder and low weight at birth according to gestational age (B)

The free amino acid levels of cubital vein plasma are compared to normal levels at the same age. Mean and s.d. are given in  $\mu\text{mol/l}$  plasma.

		n	Mean	s.d.	Significance of difference
<b>1-3 hours of age</b>					
Leucine	A	4	62	11	
	Normal	10	80	9	
	B	10	103	21	
<b>4-6 hours of age</b>					
Leucine	A	6	49	6	
	Normal	18	67	12	

branch-chained amino acid levels of plasma from 1-3 to 4-6 hours of age (Fig. 6b). In the growth retarded group the same tendency towards increased levels at 1-3 hours of age and an increased disappearance from plasma thereafter was also seen in the other essential amino acid levels studied. See Table 2.

At 4 hours of age the urea plasma level was higher than normal when pregnancy had been complicated by hypertension. Alanine and proline showed a tendency towards increased levels in the growth retarded group. In one growth retarded case where a marked neonatal hypoglycaemia with jitteriness was observed (8 mg at 12 hours of age) the increase of the alanine and proline levels of plasma was marked (Fig. 6a).

One hour after 10 g of breastmilk given at 4 hours of age the levels of leucine, proline, histidine and phenylalanine were increased in the growth retarded group (Fig. 7).

The urine collected at 4-7 hours of age showed no significant differences between the ratios of the concentrations of the different amino acids compared to the normal material. There was a tendency towards low urinary creatinine and polyuria in the hypertensive group.

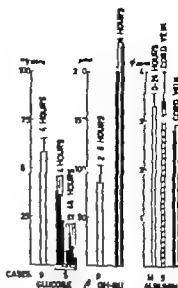


Fig. 8 Glucose and  $\beta$  hydroxybutyrate levels at 4 hours of age and cord vein albumin level. Unfilled columns represent normal mean  $\pm 2$  s.d. Filled columns represent individual levels of these investigated newborns of mothers with hypertensive disorder during pregnancy who had low birth weight for gestational age. The striped column represents cord vein plasma level  $\pm$  range of those newborns of mothers with hypertensive disorder of pregnancy who had normal birth weight for gestational age. The normal albumin level during the first day of life (mean  $\pm 2$  s.d.) is according to Sesto *et al.* (25).

Low blood glucose levels and a tendency towards higher levels of  $\beta$  hydroxybutyrate at 4 hours of age and lower plasma albumin level in cord blood of the growth retarded group is demonstrated in Fig. 8.

## DISCUSSION

The only changes of the plasma free amino acid levels found during the initial fast of the normal newborn (Fig. 3) are identical to those found in caloric deficiency. Experimental caloric undernutrition leads to a characteristic decline of the branch-chained amino acid and alanine levels of plasma while the glycine level and the ornithine/arginine quotient increase (1, 13). The lysine level decreases in experimental protein restriction in addition to the above mentioned changes of amino acid levels while the alanine level increases (1, 2, 28, 31).

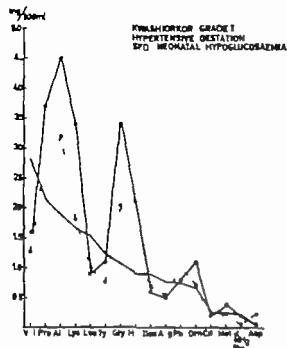


Fig 9 The plasma amino acid levels of Kwashiorkor Grade I (16) and of a newborn of mother with hypertensive disorder during pregnancy who had low birth weight and short length for gestational age and developed neonatal hypoglycaemia. Threonine, serine and tryptophan levels were not determined (19). Taurine is excluded as its foetal metabolism seems to be unique to the foetus (21). Normal levels of infants (16) are indicated by the declining solid line.

These changes in the homeostasis of free amino acids of plasma are evident less than 48 hours after the reduction of protein intake in normal infants (28).

The findings are in agreement with the demonstrated early postnatal intensification of hepatic accumulation of amino acids in the guinea pig (7) and the increased mobilization and utilization of fat during the neonatal period (23, 32). The fast urinary excretion and continuous drop of the plasma level of taurine has been discussed earlier (21). Equivalence of essential amino acid concentration in portal and radial venous blood has been demonstrated (10). Time study of cubital venous plasma in individual cases of the present investigation, from within half an hour after birth shows a continuous drop of the essential amino acid levels studied to the levels at 4 hours of age. This justifies a comparison of cord vein and 4-hour-of age cubital vein levels.

In the newborn of mothers with hypertensive disorder the resistance to decline of the alanine and proline levels, and the faster decrease of the essential amino acid levels seem to be correlated to the severity of the SFD syndrome (Fig 6). These changes are the same as those found in protein deficiency of children (3, 11, 16, 27, 33, 35). The alanine level that decreases in experimental caloric undernutrition and increases in experimental protein undernutrition also increases in the protein deficiency of Kwashiorkor Grade I (16). Splanchnic uptake of alanine greatly exceeds that of all other amino acids (6). Proline is also increased in the protein deficiency of Kwashiorkor and in experiments with low and excess protein intake in children and infants (28). The increased levels of proline seem to be characteristic of protein malnutrition of children independent of the type of malnutrition.

Apart from a more pronounced change in the hypoglycaemic growth retarded newborn (Fig 9) there also seems to be a resistance to decline of the tyrosine level. This can be explained by the fact that these children are born slightly early the known deficiency of *p*-hydroxy phenylpyruvate oxidase activity in the liver of foetuses (17) and the high levels of tyrosine found in the plasma of early borns at 4–6 hours of age (Fig 5) as well as in some babies later during the neonatal period (4, 18).

Thus the growth retarded newborns of mothers with hypertensive disorder of pregnancy showed the signs that have been called the earliest biochemical abnormality of malnutrition (34). The amino acid pattern is compatible with protein deficiency.

In the growth retarded group the first breastmilk feed of 10 g at 4 hours of age causes a one hour postprandial increase of above all the leucine level of plasma. This change is equalled in the normal fasting newborn by 30 g at 48 hours of age (Fig 7). In the normal full term newborn the amino acid levels have been reported to be unchanged one hour postprandially (15). The leucine content of breastmilk protein is among the highest for

any amino acid (26) and the branch-chained amino acids are characterized by a fast intestinal absorbance (14). The general tendency was towards a postprandial increase of the amino acid levels with fastest neonatal decline and decrease of the amino acid levels that increase during the initial fast. This means that the first feed of the growth retarded newborn is accompanied by a greater postprandial fluctuation of the free amino acid levels of plasma especially if the first feed is delayed. This might be of clinical importance as high protein intake seems to be able to aggravate the course of the hypoglycaemic attacks occasionally seen in growth retarded newborns (9).

### SUMMARY

During the first hours of extrauterine life the venous plasma of the fasting normal newborn shows a fast decline of valine, leucine, isoleucine, lysine and alanine levels while the glycine level and the ornithine/arginine quotient seem to increase. The changes are not caused by urinary excretion and are the same as those found in calorie undernutrition.

The fasting newborn of mothers with hypertension of pregnancy who are underweight and short for gestational age have increased levels of valine, leucine and isoleucine during the first hours after birth and a tendency towards a more rapid decline of the levels thereafter. The alanine and proline levels seem to resist the general decline during the initial fast. These changes in the homeostasis of plasma free amino acids are not due to short gestational period. They are the same as those found in protein deficiency.

### ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr Bengt Persson for the  $\beta$ -hydroxybutyrate determinations.

### REFERENCES

- 1 Adinolfi A. Influence of dietary deprivation on plasma concentration of free amino acids of man. *J Appl Physiol* 25: 52, 1968.

- 2 Arroyave G. The estimation of relative nutrient intake and nutritional status by biochemical methods. *Protein Am J Clin Nutr* 11: 447, 1962.
- 3 Arroyave G, Wilson D, de Ferra C & Behar M. The free amino acids in blood plasma of children with kwashiorkor and marasmus. *Am J Clin Nutr* 11: 517, 1962.
- 4 Blotman H, R. Day M, O. Gebbs N. K. & Woolf L. I. An inborn defect in the metabolism of tyrosine in infants on a normal diet. *Biochem J* 77: 320, 1960.
- 5 Butler M. R. & Bonham H. G. *Perinatal mortality*. Livingstone, Edinburgh, 1963.
- 6 Carlsson A., Hallgren B., Jagenburg R., Svenborg A. & Warkö L. Antenatal plasma deficiencies of free fatty acids and amino acids. *Acta Med Scand* 181: 199, 1967.
- 7 Christensen H. N. & Clifford J. B. Early postnatal autolysis of hepatic accumulation of amino acids. *J Biol Chem* 238: 1743, 1963.
- 8 Clouston C. A. B. & Christensen J. The placental transfer of amino acids in normal and toxemic pregnancy. *J Obstet Gynec Br Emp* 61: 364, 1954.
- 9 Cochrane W. A. Studies in the relationship of amino acids to infantile hypoglycemia. *Am J Dis Child* 99: 476, 1960.
- 10 Deaton A. E. & Elvehjem C. A. Availability of amino acids in vivo. *J Biol Chem* 206: 449, 1954.
- 11 Endonson J. C., Phillips E. J. & Collin W. R. F. The free amino acids of plasma and urine in kwashiorkor. *Lancet* 1: 615, 1960.
- 12 Engström L. & Searby G. Standardisering för vikt och längd hos nyfödda barn. *Läkarsällningen* 51: 492, 1966.
- 13 Felig P., Owen O. E., Wahren J. & Cahall G. F. Jr. Amino acid metabolism during prolonged starvation. *J Clin Invest* 43: 534, 1969.
- 14 Fries E. Q. The levels of individual free amino acids in the plasma of normal man at various intervals after a high protein meal. *J Clin Invest* 37: 1710, 1958.
- 15 Ghadami H. & Pecora P. Plasma amino acids after birth. *Pediatrics* 34: 182, 1964.
- 16 Holt, L. B., Kasperman S. E., Norrish P. M., Rotman E. & Finch J. The plasma aminoograms in kwashiorkor. *Lancet* 11: 1343, 1963.
- 17 Kretschmer N. Enzymatic pattern during development: an approach to a biochemical definition of maturity. *Pediatrics* 23: 606, 1959.
- 18 Levine S. Z., Margolis E. & Cordon H. H. A defect in the metabolism of tyrosine and phenylalanine in premature infants. *J Clin Invest* 20: 199, 1941.
- 19 Lindblad B. S. & Balderston A. The normal venous plasma free amino acid levels of non-pregnant women and of mother and child during delivery. *Acta Paediatr Scand* 36: 37, 1967.
- 20 Lindblad B. S. & Zetterstrom R. The venous plasma free amino acid levels of mother and child during delivery II. After short gestation and gestation complicated by hypertension with



- special reference to the small for dates syndrome *Acta Paediat Scand* 57 195 1968
- 21 Lindblad B & Baldesten A Time studies on free amino acid levels of venous plasma during the neonatal period *Acta Paediat Scand* 58 252 1969
  - 22 Marks V An improved glucose oxidase method for determining blood CSF and urine glucose *Clin Chim Acta* 4 395 1959
  - 23 Persson B & Gentz J The pattern of blood lipids, glycerol and ketone bodies during the neonatal period infancy and childhood *Acta Paediat Scand* 55 353 1966
  - 24 Persson B Determination of plasma acetoacetate and  $\alpha\beta$  hydroxybutyrate in newborn infants by an enzymatic fluorimetric micromethod To be published *Scand J Clin Lab Invest* 1969
  - 25 Sato M Gittleman I F Pincus J B & Sobel A E Plasma protein patterns in premature infants of varying weights on the first day of life *Pediatrics* 17 657 1956
  - 26 Saito & Furuechi *Studies on human milk* Research laboratory Snow Brand Milk Products Co 3-21 Akabanchi, Kitaku Tokyo 1965
  - 27 Saunders S J Truswell A S Brubeck G O Wittman W & Hansen J D L Plasma free amino acid pattern in protein-calorie malnutrition Reappraisal of its diagnostic value *Lancet* II 795 1967
  - 28 Snyderman S E Holt L E Jr Norton P M Rortman E & Phatak S V The plasma aminoogram I Influence of the level of protein intake and a comparison of whole protein and amino acid diets *Ped Res* 2 131 1968
  - 29 Soupart P Free amino acids of blood and urine in the human In J T Holden (ed) *Amino acid pools* Elsevier Press Inc New York 1962
  - 30 Spinco Division *Electrophoresis on cellulose acetate membranes in the microzone system* Beckman Instruments Inc Palo Alto California 1969
  - 31 Swendsen M E Griffith W H & Tuttle S G The effect of a low protein diet on the ratio of essential to nonessential amino acids in blood plasma *Metabolism* 12 96 1963
  - 32 Van Duyn C Parker H & Holm L Metabolism of free fatty acids during perinatal life of lambs *Am J Obstet* 91 277 1965
  - 33 Westall R G Rortman E de la Pena C Rasmussen H Cravioto J Gomez F & Holt L E Jr The plasma amino acids in malnutrition *Arch Dis Child* 33 499 1958
  - 34 Whitehead R G Rapid determination of some plasma amino acids in subclinical kwashiorkor *Lancet* I 250 1964
  - 35 Whitehead R G & Dean R F A Serum amino acids in kwashiorkor I Relationship to clinical condition *Am J Clin Nutr* 14 313 1964
  - 36 Wigglesworth J S Foetal growth retardation *Br Med Bull* 22 13 1966

Submitted Sept 11 1969

Accepted Sept 11 1969

Dept of Paediatrics  
Kronprinsessan Lovisas Barnsjukhus  
Polhemsgatan 30  
112 30 Stockholm  
Sweden

**Key words** Amino acids newborn infants small for dates syndrome gestational complications

## THE VENOUS PLASMA FREE AMINO ACID LEVELS DURING THE FIRST HOURS OF LIFE

*II In a Lower Socio economic Group of a Refugee Area in Karachi  
West Pakistan with Special Reference to the Small for Dates Syndrome*

B S LINDBLAD RAZIA J RAHIMTOOLA and NAFEEZ KHAN

*From the Department of Paediatrics at Crown Princess Lovisa's Children's Hospital  
and the Department of Chemistry II Karolinska Institute Stockholm Sweden and the  
Department of Paediatrics Jinnah Postgraduate Medical Centre Karachi West Pakistan*

The marked differences in birth weight between different races seems to be largely dependent on socio-economical differences (10). Since protein undernutrition is common in many economically developing countries the question arises if protein undernutrition is of significance to the lower birth weight seen in these areas (9, 19). Protein undernutrition of the pregnant rat and dog leads to growth retardation and late neurological sequelae of the offspring (5, 12, 18, 21).

Certain changes in the homeostases of plasma free amino acids are characteristic of protein deficiency in the human (3, 6, 17, 20, 25, 26). The aim of the present investigation is to find out whether the babies of a low socio-economical group of West Pakistani women show these signs of protein deficiency. The free amino acid levels of cord vein plasma of this socio-economical group have been reported earlier (16). The plasma levels of free amino acids during the immediate postnatal period of Swedish normal early born and growth retarded newborns have been given in a previous paper (17).

This work was made possible through grants from AB Sanner. Fund for Nutritional Research and grant no. 383 from the Swedish Medical Research Council.

## MATERIAL

The group investigated is the low socio-economical group more extensively described earlier (16). The mothers were nutritionally characterized by daily low consumption of vegetable protein during both the premenstrual as well as maternal stage. Their venous plasma at the time of delivery had the increased glycine level and glycine/valine quotient characteristic of protein deficiency. Among the infants of this group there was a greater frequency of low birth weight and short length for gestational age than in a middle-class group with the same racial and cultural background when the birth weights and lengths of both groups were compared to an "ideal" standard (7). Length of pregnancy given during consultation with the mother was 38-41 weeks. The newborn showed no perinatal signs or feeding difficulties typical of short pregnancy upon investigation during the 3-day postnatal care. There was no hypertension of the mothers at the time of delivery and no signs of intra- or extruterine asphyxia.

## METHODS

The experimental conditions were identical to those of the normal material (17) except that during the experimental period the babies in the present investigation were kept covered with a quilt in bed and not in an incubator.

Collection of samples, storage and deproteinization has been described earlier (14). In 7 cases a complete analysis of all neutral and acidic amino acid and urea levels at 4 hours of age was made on 0.5-1.0 ml plasma with a Beckman 120 B automatic amino acid analyzer (14). In all 21 cases neutral essential amino acids were determined in samples taken at different times during the first 6 hours of life. 0.3 ml of plasma

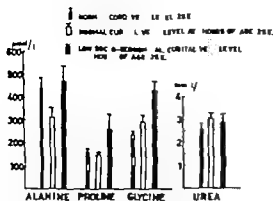


Fig 1 Differences from the normal free amino acid levels of plasma at 4 hours of age

was used according to the method described in an earlier report (15)

Plasma protein levels were determined by electrophoresis on cellulose acetate (23)

## RESULTS

At 4 hours of age there was a significant increase of the alanine, proline, glycine and taurine levels compared to the normal levels at this age (Fig 1). The branch chained amino acid levels of plasma showed a delay in their normal decline (Fig 2). The other amino acids investigated showed normal plasma levels.

Cord vein plasma concentrations of protein components were normal.

## DISCUSSION

The increased plasma levels of alanine, proline and glycine found are characteristic of

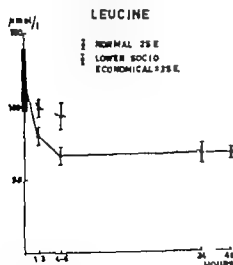


Fig 2 Leucine plasma levels during the immediate neonatal period

Table 1 Free amino acid levels of cubital vein plasma compared to normal levels at the same age (17)

Mean and s.d. are given in μmol/l plasma. \* = significant 0.1 < p < 1. \*\* = highly significant p < 0.1

	n	Mean	s.d.	Significance of difference
<b>1-3 hours of age</b>				
Leucine	20	99	14	*
Normal	10	80	9	
<b>4-6 hours of age</b>				
Leucine	21	94	20	**
Normal	18	67	12	
<b>4 hours of age</b>				
Glycine	7	428	57	**
Normal	9	293	48	
Alanine	7	469	95	**
Normal	9	311	64	
Proline	7	261	93	**
Normal	9	147	24	
<b>Cord vein levels (16)</b>				
Glycine	9	320	57	
Normal (14)	10	239	32	
Proline	9	210	36	**
Normal	10	160	28	

protein deficiency in infants and children, both after experimental protein restriction (1, 2, 22, 24) and in the clinical syndrome of Kwashiorkor Grade I (11).

A temporary increase of the branch chained amino acid levels of plasma with a rapid fall thereafter is reported to occur in experimental starvation of adults (1, 8) and was found in newborn small for dates (undue low birth weight for gestational age) of mothers with hypertensive disorder of pregnancy (17). Normal urea, tyrosine and phenylalanine levels contradict the possibility of an increased peripheral release of amino acids in the newborns (4). The findings are not compatible with short pregnancy (13, 17).

The taurine level was increased at 4 hours of age compared to the normal at this age. Increased taurine level has been reported in

kwasinhorkor Grade I (22). As the normal foetal metabolism of taurine seems to be unique to the foetus (15) no conclusions can be drawn from this finding.

The increased levels of glycine and the gly cine/valine quotient, typical of the calorie deficiency of the first fasting period of the normal newborn (17) and of proline characteristic of protein malnutrition of infants (22) were seen already in cord ven plasma of this socio-economical group (16). See Table 1. The alanine level normally declines in plasma rapidly after birth (17). The resistance to decline of alanine found in this group is not compatible with calorie deficiency (1-8). The findings suggest a protein deficiency starting during intrauterine life.

### SUMMARY

The plasma aminogram was investigated in newborns from a low socio-economical group of West Pakistan with an increased frequency of the small for dates syndrome.

The plasma levels of proline and glycine increased during the first hours of life to levels significantly higher than those of a Swedish normal material. The alanine levels showed a resistance to decline during this period. The branch-chained amino acid levels of plasma showed a fast decline after a temporary resistance to decline.

The plasma free amino acid levels changed during the first hours of life to a pattern which is the same as that seen in protein deficiency.

### ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Prof. Dr. Hamid Ali Khan for his kind interest in the investigation, to Professor Azeel Rehman and Dr. A. H. Haque for helpful discussions and to Dr. Tofayel Ahmed for his kind support.

### REFERENCES

1. Adibi S A. Influence of dietary deprivation on plasma concentration of free amino acids of man. *J Appl Physiol* 23: 52, 1968.

2. Arroyave H. The estimation of relative nutrient intake and nutritional status by biochemical methods. *Protein Am J Clin Nutr* 11: 447, 1962.
3. Arroyave G, Wilson D, de Funes C & Behar M. The free amino acids in blood plasma of children with kwashiorkor and marasmus. *Am J Clin Nutr* 11: 517, 1962.
4. Bremer H J & Neumann W. Phenylalanine toleranz bei Frühgeborenen reifen Neugeborenen Säuglingen und Erwachsenen. *Klin Wochschr* 44: 1076, 1966.
5. Chow B F & Lee C J. Effect of dietary restriction of pregnant rats on body weight gain of the offspring. *J Nutr* 82: 10, 1964.
6. Endozen, J C, Phillips L J & Collie, W R. F. The free amino acids of plasma and urine in kwashiorkor. *Lancet* 1: 615, 1960.
7. Engström L & Starck O. Standardkurvor för vikt och längd hos utlödda barn. *Läkarsamfundet* 31: 49, 1966.
8. Fehg P, Owen O E, Wahren J & Cahill G F Jr. Amino acid metabolism during prolonged starvation. *J Clin Invest* 48: 584, 1969.
9. Ghosh S & Daga, S. Comparison of gestational age and weight in standards of prematurity. *J Pediatr* 71: 173, 1967.
10. Gruenewald P. Growth pattern of the normal and the deprived fetus. In J H P Jorvis H K, A Voser & J A Trostira (eds). *Aspects of prematurity and dysmaturity*. Nutricia Symposium. H E Steinfert Kroese N V, Leiden, 1968.
11. Holt L E, Strydom S E, Norton P M, Rotman E & Finch J. The plasma aminogram in kwashiorkor. *Lancet* II: 1341, 1963.
12. Haseh A M, Agoston C E & Chow B F. Growth of young rats after differential manipulation of maternal diet. *J Nutr* 91: 195, 1967.
13. Kretschmer N. Enzymatic pattern during development: an approach to a biochemical definition of immaturity. *Pediatrics* 23: 606, 1959.
14. Lindblad B S & Baldesten A. The normal venous plasma free amino acid levels of non-pregnant women and of mother and child during delivery. *Acta Paediatr Scand* 38: 37, 1967.
15. Lindblad B S & Baldesten A. Time studies on free amino acid levels of venous plasma during the neonatal period. *Acta Paediatr Scand* 38: 252, 1969.
16. Lindblad B S, Rahemtola R J, Sand M, Haque Q & Khan N. The venous plasma free amino acid levels of mother and child during delivery III. In a lower socio-economic group of a refugee area in Karachi, West Pakistan with special reference to the small for dates syndrome. *Acta Paediatr Scand* 58: 497, 1969.
17. Lindblad B S. The venous plasma free amino acid levels during the first hours of life. After normal and short gestation and gestation complicated by hypertension. With special reference to the small for dates syndrome. *Acta Paediatr Scand* 59: XXX, 1970.

18 Platt H S Experimental protein-calorie deficiency in R A McCance & Elsie M Widdowson (eds) *Calorie deficiencies and protein deficiencies* J A Churchill Ltd London 1968

19 Rahimtoola R J Mir S & Baloch S Low birth weight the small for dates syndrome and perinatal mortality in a low family income group *Acta Paediat Scand* 57 534 1968

20 Saunders H J Truswell A S Barbezat G O Wittman W & Hansen J D L Plasma free amino acid pattern in protein-calorie malnutrition Reappraisal of its diagnostic value *Lancet* II 795 1967

21 Simonson M Yu W Anilane J K Sherwin R & Chow H F Studies of development in progeny of underfed mother rats *Fed Proc* 26 519 1967

22 Snyderman S E Holt L E Jr Norton P M Rostman E & Phansalkar S V The plasma aminogram I Influence of the level of protein intake and a comparison of whole protein and amino acid diets *Ped Res* 2 131 1968

23 Spanco Division *Electrophoresis on cellulose acetate membranes in the micro zone system* Beckman Instruments Inc Palo Alto California 1969

24 Swendsen M E Griffith W H & Tuttle S G The effect of a low protein diet on the ratio of essential to nonessential amino acids in blood plasma *Metabolism* 12 96 1963

25 Westall R G Rostman E de la Pena C Rasmussen H Cravioto J Gomez F & Holt L E Jr The plasma amino acids in malnutrition *Arch Dis Child* 33 499 1958

26 Whitehead R G & Dean R F A Serum amino acids in kwashiorkor I Relationship to clinical condition *Am J Clin Nutr* 14 313 1964

Submitted Sept 9 1969  
Accepted Sept 10 1969

(B S L.) Dept of Paediatrics  
Kronprinsessan Lovisas Barnsjukhus  
Polhemsgatan 30  
112 30 Stockholm  
Sweden

**Key words** Amino acids plasma newborn infants small for dates syndrome lower socio-economic groups

## C REACTIVE PROTEIN IN SERUM FROM INFANTS AS DETERMINED WITH IMMUNODIFFUSION TECHNIQUES

### 1 Healthy infants

J. SAXSTAD, L. A. NILSSON and L. A. HANSON

From the Department of Paediatrics and Institute of Medical Microbiology  
Departments of Bacteriology and of Immunology University of Göteborg Göteborg  
Sweden

Earlier clinical studies of C reactive protein (CRP) in serum have usually been performed with the capillary tube precipitation method (1). The results obtained with this method vary considerably due to differences in the antisera (4). Investigations using immunodiffusion techniques have shown a greater sensitivity and accuracy of the determination of CRP (5-7). Sera from infants have not yet been studied with these methods. A test which can be used as an indicator of infection is of special interest in this age group since here the usual clinical and laboratory criteria for infection are often less reliable—especially in the young infants. Felix *et al* (3) reported that with the capillary tube precipitation technique one third of their infected infants had no demonstrable CRP in their sera. It should be of interest to see whether or not the more sensitive immunodiffusion methods could detect CRP in more of these infected cases and if the CRP concentration in serum corresponds to the clinical condition. As a basis for such an evaluation of the possible clinical usefulness of these methods for the detection of CRP in this age a group of normal infants was studied.

### MATERIAL AND METHODS

Capillary blood samples were collected from 100 infants from 1-15 months of age on their regular

health controls at the child welfare clinic. Any pathological findings on the clinical examination were recorded. Infants with demonstrable CRP were taken to it for repeated blood sampling and renewed clinical examination.

The comparative double diffusion in gel technique was used for the detection of CRP (5-7). The sensitivity limit of this technique is about 0.5 µg/ml. Positive samples were quantitated with the single radial immunodiffusion technique as earlier described (5). This method detects concentrations exceeding 1 µg/ml. Concentrations between 0.5 and 1 µg CRP/ml are recorded as trace amounts. Commercially available anti-CRP from sheep and goat (Hyland Lab. Los Angeles, California) was used.

### RESULTS

The age distribution of the infants is illustrated in Fig. 1 where the CRP containing sera are also indicated. There is no evident relation between age and the frequency of CRP containing samples as judged from these small groups.

The presence of CRP correlated to the clinical findings is recorded in Table 1. Ninety eight of the 100 infants were healthy on clinical examination. In seventeen of these apparently healthy infants CRP was found although in thirteen of the sera it was merely present in trace amounts detectable only with the double diffusion method and not with the quantitative radial diffusion technique. Four of these infants had suffered from upper respiratory tract infections within a week before

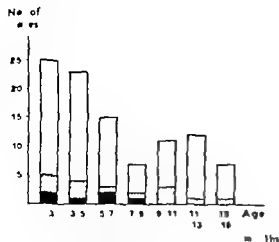


Fig 1 Number of sera containing CRP related to age □ CRP not demonstrable ▨ trace amounts of CRP ■ CRP > 1 µg/ml

the sampling and recovered with no detectable CRP on reexamination. The history was otherwise uneventful in these thirteen infants as well as in the four cases where CRP was quantifiable (11, 6, 3 and 2 µg/ml). The infant with the highest amount of CRP that had been apparently healthy upon examination was two weeks later found to have pyelonephritis and anatomical abnormalities of the kidneys. At that time it still had CRP in its serum. The three other of the four with CRP concentrations in serum exceeding 1 µg/ml had no demonstrable CRP upon check-up.

The two of the 100 infants which had positive findings on clinical examination (bronchitis) both had CRP in their sera, one with trace amounts and one with 16 µg/ml.

Table 1 Relation between the presence or absence of CRP and the result of clinical examination

CRP		Clinical examination		Total
		Positive	Negative	
Present	> 1 µg/ml	1	4	5
	trace amounts (0.5-1 µg/ml)	1	13	14
Not demonstrable		0	81	81
Total		2	98	100

In healthy infants Andersen *et al* (2) and Felix *et al* (3) showed a frequency of CRP in serum increasing to above 50% during the first week of life. With increasing age Felix *et al* found CRP in decreasing frequencies down to 2% in healthy infants 1-6 months of age. With the more sensitive and accurate technique used in this study CRP could be demonstrated in 17 (17%) of the infants apparently healthy on their visit to the baby welfare clinic. Thirteen of these (13) only had CRP in trace amounts. This group included four infants in whom the positive finding could presumably be related to a recent upper respiratory infection. The only case where CRP had not vanished on reexamination turned out to have kidney abnormalities and pyelonephritis.

In a random sample of 50 year-old men CRP was demonstrable in a frequency of 75% with the methods used in this study, mostly only in trace amounts, however (8). In a group of blood donors lower frequencies of sera containing trace amounts of CRP were found with lower age (6). In the infants of this study CRP was demonstrable in 19%. These CRP-positive sera usually only contained trace amounts and at present this cannot be related to any clinical findings but for the few cases with recent upper respiratory infections.

The results from this material of healthy infants may be used as a basis for a study of the clinical implications of the CRP concentration in diseased infants. Such a study has been performed (9).

## SUMMARY

Using sensitive and accurate immunodiffusion methods CRP could be demonstrated in 17 of 98 infants apparently healthy upon clinical examination. Thirteen of these sera only contained trace amounts of CRP (0.5-1 µg/ml). The only case which had quantifiable amounts of CRP and which did not become negative upon control turned out to have kidney abnormalities and pyelonephritis.

## REFERENCES

- 1 Anderson H C & MacCarty M Determination of C reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever *Amer J Med B* 445 1950
- 2 Anderson H J, Hanson, L. A & Nilsson, L. A The presence of C reactive protein in the neonatal period correlated to clinical conditions *Acta Path Microbiol Scand* 54 349 1962 (Abstract)
- 3 Felix N S, Nakajima, H & Kagan B M Serum C reactive protein in infections during the first six months of life *Pediatrics* 37 270 1966
- 4 Nakamura R M, Maggiasy R, Ford J & Kunkel G M Significant variations in commercial C reactive protein antisera. *Amer J Clin Path* 44 290 1965
- 5 Nilsson L. A. Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum *Acta Path Microbiol Scand* 73 129 1968
- 6 — C reactive protein in apparently healthy individuals (blood donors) related to age *Acta Path Microbiol Scand* 73 619 1968
- 7 Nilsson L. A. & Hanson L. A. Studies on C reactive protein. I. Demonstration of C reactive protein by precipitation in capillary tubes and by precipitation in agar gel *Acta Path Microbiol Scand* 54 335 1962
- 8 Nilsson, L. A. & Tibblin G. C reactive protein in a random sample of Swedish men aged fifty. Distribution and relation to clinical manifestations *Acta Med Scand* 183 467 1968
- 9 Saxstad J, Nilsson L. A. & Hanson L. A. C reactive protein in serum from infants as determined with immunodiffusion techniques. II. Infants with various infections. In manuscript

Submitted July 12 1969

Accepted Aug 9 1969

(L. A. H.) Dept. of Immunology  
Inst. of Medical Microbiology  
Göteborg  
Sweden

**Key words** C reactive protein healthy infants immunodiffusion techniques



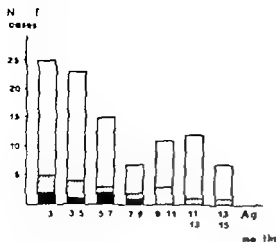


Fig 1 Number of sera containing CRP related to age □ CRP not demonstrable /// trace amounts of CRP ■ CRP > 1 µg/ml

the sampling and recovered with no detectable CRP on reexamination. The history was otherwise uneventful in these thirteen infants as well as in the four cases where CRP was quantifiable (11, 6, 3 and 2 µg/ml). The infant with the highest amount of CRP that had been apparently healthy upon examination was two weeks later found to have pyelonephritis and anatomical abnormalities of the kidneys. At that time it still had CRP in its serum. The three other of the four with CRP concentrations in serum exceeding 1 µg/ml had no demonstrable CRP upon check up.

The two of the 100 infants which had positive findings on clinical examination (bronchitis) both had CRP in their sera: one with trace amounts and one with 16 µg/ml.

Table 1 Relation between the presence or absence of CRP and the result of clinical examination

CRP		Clinical examination		Total
		Positive	Negative	
Present	> 1 µg/ml	1	4	5
	trace amounts (0.5-1 µg/ml)	1	13	14
Not demonstrable		0	81	81
Total		2	98	100

## DISCUSSION

In healthy infants Andersen *et al* (2) and Felix *et al* (3) showed a frequency of CRP in serum increasing to above 50% during the first week of life. With increasing age Felix *et al* found CRP in decreasing frequencies down to 2% in healthy infants 1-6 months of age. With the more sensitive and accurate technique used in this study CRP could be demonstrated in 17 (17%) of the infants apparently healthy on their visit to the baby welfare clinic. Thirteen of these (13%) only had CRP in trace amounts. This group included four infants in whom the positive finding could presumably be related to a recent upper respiratory infection. The only case where CRP had not vanished on re-examination turned out to have kidney abnormalities and pyelonephritis.

In a random sample of 50 year old men CRP was demonstrable in a frequency of 75% with the methods used in this study, mostly only in trace amounts, however (8). In a group of blood donors lower frequencies of sera containing trace amounts of CRP were found with lower age (6). In the infants of this study CRP was demonstrable in 19%. These CRP positive sera usually only contained trace amounts and at present this cannot be related to any clinical findings but for the few cases with recent upper respiratory infections.

The results from this material of healthy infants may be used as a basis for a study of the clinical implications of the CRP concentration in diseased infants. Such a study has been performed (9).

## SUMMARY

Using sensitive and accurate immunodiffusion methods CRP could be demonstrated in 17 of 98 infants apparently healthy upon clinical examination. Thirteen of these sera only contained trace amounts of CRP (0.5-1 µg/ml). The only case which had quantifiable amounts of CRP and which did not become negative upon control turned out to have kidney abnormalities and pyelonephritis.

Table 1 Serum amino acid levels ( $\mu\text{moles/l}$ )

Age (days)	Normal range				
	3	6	22	Newborn 1-3 days	Infants <sup>a</sup>
Daily protein intake (g/kg b.w.)	1.5	0.2	3.2		
Threonine	360	300	210	74-216	19-91
Threonine	1890	730	240	114-335	
Glutamic acid	450	460	550	20-107	46-290
Glycine	850	810	950	224-514	56-308
Alanine	300	420	260	236-410	99-313
Valine	450	720	270	80-246	57-262
Methionine	Trace	80	40	9-41	3-39
Isoleucine	200	290	110	27-94	26-84
Leucine	340	530	270	47-103	45-155
Tyrosine	400	200	90	42-99	11-122
Phenylalanine		190	90	42-110	23-69
Ornithine	480	430	200	49-151	
Lysine	710	830	360	114-269	45-144
Histidine	270	260	70	49-114	
Arginine	210	260	60	22-88	

Dickinson *et al.* Pediatrics 36 2, 1965 (3)  
 Soupart 1962 (15)

## METHODS

Serum and urine samples for the amino acid analyses were collected before and during the dietary treatment and stored at  $-20^{\circ}\text{C}$  from 2 weeks to 3 months before analyzed. The pH of the urine samples varied between 5-6. For screening and semiquantitative purposes high voltage electrophoresis and paper chromatography were used. Quantitative analyses were performed on a Technicon Amino Acid Analyzer after precipitation of the serum proteins with sulfosalicylic acid (50 mg/ml serum). Threonine and glutamine were well separated in these analyses while serine and glutamate were not equally well separated. Proline and hydroxyproline were not measured. Due to the storage the serum values of glutamic acid are not reliable (3). The serum levels of amino acids are expressed as  $\mu\text{moles/l}$  and the urinary excretion as  $\mu\text{moles/mg}$  of creatinine.

## RESULTS

In Table 1 are listed the serum amino acid values of the patient on days 3, 6 and 22. The protein intake on the preceding days are also given. On the third and sixth day of life the patient showed an almost generalized hyperaminoacidemia. Most increased were the levels of glycine, threonine, lysine, serine, valine, leucine, isoleucine and glutamic acid. On the 22nd

day the pattern was changed with most of the serum amino acids within or slightly above the normal range. Glycine was however still markedly elevated, so were to a lesser extent also leucine and lysine.

A high voltage electrophoresis and paper chromatography of urine collected when the infant was 24 hours old was normal for age. Table 2 illustrates the urinary excretion of amino acid on days 3, 6 and 18. On the 3rd and 6th day there was a slight generalized hyperaminoacidemia with glycine and lysine showing the most marked elevation. The excretion of valine and threonine was moderately increased. On the 18th day there was a more isolated increase in the excretion of glycine, threonine, valine, lysine and ornithine.

## DISCUSSION

At birth the amino acid patterns of urine from our patient seemed to be normal as judged by semiquantitative methods. The abnormal changes soon occurred however. Already on the third day of life when the clinical symptoms became apparent, an almost generalized

## ABNORMAL PATTERNS OF URINE AND SERUM AMINO ACIDS IN METHYLMALONIC ACIDEMIA

S HALVORSEN O STOKKE and L ELDJARN

*From the Pediatric Research Institute and the Institute of Clinical Biochemistry  
University of Oslo Rikshospitalet Oslo Norway*

Methylmalonic acidemia (MMA emia) is a recently discovered inborn error of metabolism independently described by Oberholzer *et al* (10) in a 6 year old girl and by Stokke *et al* (16) in a newborn infant. The disease is clinically characterized by acidosis, dehydration, lethargy and coma. High serum levels and a large urinary excretion of methylmalonic acid (MMA) are characteristic chemical findings.

MMA is normally formed by carboxylation of propionyl CoA. The latter compound arises from many sources. The amino acid isoleucine, threonine and methionine are probably the most important in quantity but propionate is also formed from odd and branched chain fatty acids, thymine,  $\beta$  alanine, uracil and cholesterol. It is uncertain whether valine is metabolized through propionyl CoA or to methylmalonyl CoA directly. Two different enzymatic steps are involved in the conversion of methylmalonyl CoA to succinyl CoA: an isomerization of MMA CoA catalyzed by an isomerase followed by an intramolecular rearrangement catalyzed by a coenzyme B<sub>12</sub> dependent mutase. It has been suggested that the metabolic block in MMA-emia is found in the latter step (10) in some cases possibly due to a decreased action of the vitamin B<sub>12</sub> cofactor (12).

A distinct finding in MMA emia is high serum levels of glycine (11, 16). In this paper the amino acid disturbances in our case of MMA emia are described and the results are

compared with those found in Hyperglycinemia.

### CASE REPORT

In September 1967 a newborn girl developed acidosis with severe clinical symptoms and accumulation of large amounts of an unknown organic in serum and urine. This acid was identified as methylmalonic acid. We therefore named the disorder Methylmalonic acidemia.

The parents of the patient were unrelated. Two siblings, both girls, died in the neonatal period suffering from acidosis. They were both born post term. The patient appeared well at birth and was started on a regular feeding schedule with cow's milk providing up to 4 g of protein and 200 calories on the second and third day. An acidosis with a standard bicarbonate of 14 mEq/l developed on the second and third day and sodium bicarbonate had to be given. The family history and the clinical picture aroused suspicion of an inborn error of metabolism. In order to try to improve the general condition of the child the protein intake was reduced considerably from the 4th to the 6th day and the patient was maintained mainly on i.v. fluids. However, no clinical improvement occurred. From the 6th day of life the daily protein intake was gradually increased to 6-8 g or 3-4 g per kg of body weight. The body weight was steadily decreasing and the patient appeared dehydrated in spite of a fluid intake of about 200 ml/kg body weight daily.

After the accumulated organic acid had been identified as MMA an attempt to treat the infant with a diet low in valine, isoleucine, threonine and methionine was performed. This treatment reduced the serum level of MMA from 30 to about 3 mg/100 ml and the daily urinary excretion of MMA from 1 g to 150 mg. Simultaneously the clinical condition improved considerably. However, the infant who at that time suffered from an urinary tract infection developed septicemia and died 6 weeks old.

We have previously pointed out that the clinical condition and the elevated amounts of glycine in blood and urine of our child with MMA-emia closely resembled the findings described in the ketotic form of Hyperglycinemia (12). We therefore proposed a relationship between the two diseases (4, 16). The serum and urine amino acid patterns in Hyperglycinemia vary considerably with the severity of the disease. Characteristic findings are increased serum levels of glycine, serine, leucine, valine, threonine, isoleucine, glutamic acid and probably also the basic amino acids (14). Correspondingly it has also been found increased urinary levels of glycine, serine, alanine, phenylalanine, lysine, ornithine and histidine.

In fact, recent reports confirm that some cases previously diagnosed as Hyperglycinemia actually are suffering from MMA-emia (8, 9). On the other hand Rosenberg *et al* (11) and Morrow *et al* (9) did not find any accumulation of MMA in other patients with Hyperglycinemia. Recent studies on leucocytes from a patient with ketotic hyperglycinemia showed a reduced oxidation of propionate to carbon dioxide while they could degrade methylmalonate normally (7). This indicates that ketotic hyperglycinemia may be divided into methylmalonic acidemia and propionic acidemia, each of them consisting of different entities (12, 16). It is more appropriate to use these terms instead of the non specific term hyperglycinemia.

## SUMMARY

Abnormal patterns of serum and urine amino acids were found in our patient with the recently discovered inborn error Methylmalonic acidemia. The most distinct findings were increased serum and urine concentrations of lysine and glycine. In addition the serum levels of leucine, isoleucine, valine, threonine and glutamic acid and the urinary excretion of valine, threonine and ornithine were increased. Both the clinical picture and the amino acid patterns resemble those found in the ketotic

form of Hyperglycinemia. Recent studies indicate that hyperglycinemia consists of at least two separate diseases: methylmalonic acidemia and propionic acidemia.

## REFERENCES

1. Armstrong N D, Yates K N & Connelly I P. Amino acid excretion of newborn infants during the first twenty four hours of life. *Pediatr* 33 975 1964.
2. Chablis B, Nyhan W L, Borden M, Bird L & Cooke R E. Idiopathic hyperglycinemia and hyperglycinuria. A new disorder of amino acid metabolism. *Pediatrics* 27 522 1961.
3. Dickinson I C, Rosebloom H & Hamiton P B. Ion exchange chromatography of the free amino acids in the plasma of the newborn infant. *Pediatrics* 36 2 1965.
4. Halvorsen S, Stokke O & Eldjarn L. Methylmalonic acidemia/hyperglycinemia. *Lancet* i 756 1968.
5. Halvorsen, S. Unpublished observations.
6. Holt E L, Snyderman S F, Norton P M, Rosman E & Finch J. The plasma aminogram in a newborn. *Lancet* ii 1343 1963.
7. Hsu Y E, Sally K J & Rosenberg L. II. Defective propionate carboxylation in ketotic hyperglycinemia. *Lancet* i 757 1969.
8. Lindblad B, Lindblad B S, Olm P, Svanberg B & Zetterstrom R. Methylmalonic acidemia. A disorder associated with acidosis, hyperglycinemia and hyperlactatemia. *Acta Paediatr Scand* 57 417 1968.
9. Morrow G, Barnes L, Auerbach, V H & DeGeorge A M. Intermittent glycinemia and methylmalonic acidemia—a new syndrome. *The Society for Pediatric Research Atlantic City 1968 Abstract* p 20.
10. Oberholzer V G, Levin B, Burgess E Ann & Young W. Methylmalonic acidemia. An inborn error of metabolism leading to chronic metabolic acidosis. *Arch Dis Child* 42 492 1967.
11. Rosenberg L E, Liljequist, A-Ch & Hsu Y E. Methylmalonic acidemia. An inborn error leading to acidosis, long-chain ketonuria and an intermittent hyperglycinemia. *New Eng J Med* 278 1319 1968.
12. —. Methylmalonic acidemia. Metabolic block localization and vitamin B dependency. *Science* 162 803 1968.
13. Snyderman S E, Holt E L, Norton P M, Rosman, E & Pharesalter S V. The plasma aminogram. I. Influence of the level of protein intake and a comparison of whole protein and amino acid diets. *Pediatr Res* 2 131 1968.
14. Soriano J R, Teitz L S, Finberg L & Edelmann C M, Jr. Hyperglycinemia with ketonacidosis and leukonuria. Metabolic studies on the nature of the defect. *Pediatrics* 39 818 1967.

Table 2 *Urinary amino acid excretion ( $\mu$ moles/mg creatinine)*

Age (days)	3	6	18	Normal range for newborn
Daily protein intake (g/kg/b w)	1.5	0.2	3.0	
Taurine	2.02	1.25	0.78	0.20-7.64
Threonine	1.31	8.4	4.55	0.10-0.34
Glutamic acid	2.04	0.80	0.78	0-0.36
Glycine	15.7	24.5	35.6	0.16-4.46
Alanine	1.20	1.60	2.64	Trace-0.80
Valine	1.15	5.19	4.28	0-0.28
Methionine	Trace		0.51	0-0.18
Isoleucine		0.41		0-0.26
Leucine	0.74	1.69	0.01	Trace-0.14
Tyrosine	1.76	0.76	0.86	0-0.06
Phenylalanine	3.23	2.34	0.51	0-0.08
Ornithine	2.98	3.79	4.63	Trace-0.02
Lysine	14.7	18.4	12.09	0.03-0.64
Histidine	4.40	7.50	2.97	Trace-1.16
Arginine	0.55	0.84		0.02-0.30
Asparagine		5.05		0-0.12

Armstrong *et al.* Pediatrics 33:975 1964 (1)

hyperaminoacidemia and hyperaminoaciduria were manifest.

Later at the age of 3 weeks more isolated deviations from the normal patterns occurred. In serum were found increased amounts of glycine, glutamic acid, the branched acids isoleucine and leucine and the basic acids lysine and ornithine. Increased amounts of glycine, alanine, threonine, valine, lysine and ornithine were excreted in urine.

The high values for both serum and urine amino acids on the third and 6th day may indicate that the aminoaciduria was mainly of the overflow type. After this time our data do not allow any conclusion whether the increased urinary amino acid levels were due to overflow or to renal damage.

The increased levels of valine, threonine and isoleucine may easily be explained as these amino acids are precursors of MMA. The increased concentration of glutamic acid at the expense of glutamine may be due to the storage of the serum and urine samples at  $-20^{\circ}\text{C}$  (see ref. 3). The causes of the increased serum concentrations of glycine, leucine and lysine are, however, not known.

Increased excretion of lysine has been ob-

served in metabolic acidosis in the newborn and the amount may exceed the excretion of glycine which is normally the dominating amino acid also in this age period (5). In other acidotic cases the disturbances of the amino acid patterns have differed with the different underlying diseases. It is therefore unlikely that the metabolic acidosis *per se* has led to the changes found in our patient.

The weight of our patient was steadily decreasing in the period before the dietary treatment. This loss of weight may be taken as a sign of a deranged protein metabolism. The plasma aminoograms of infants suffering from kwashiorkor show increased levels of glycine and serine while the levels of the branched amino acids and of tyrosine are depressed (6). The same pattern has been found in infants given a protein deficient diet for a few weeks (13). The abnormal amino acid picture of our patient may therefore only partly be caused by the general disturbance of the protein synthesis. In addition the accumulated MMA or some of the preceding intermediates may have toxic effects on protein metabolism leading to high serum and urine concentrations of some of the amino acids.

## FIBRINOLYSIS IN NEWBORNS

H ENELUND, ULLA HEDNER and INGA MARIE NILSSON

*From the Coagulation Laboratory and the Department of Pediatrics, General Hospital, Malmö, Sweden*

Fibrinolytic activity in newborns has been studied since the beginning of the 1950s. Since that time several papers have appeared on the subject.

The interest in fibrinolysis during the neonatal period has hitherto been centred largely upon the assumption that a disturbance of fibrinolytic activity plays an etiological role in the hyaline membrane disease. A comprehensive review has recently been given by Markarian *et al.* (37). They stress the fact that the results of most investigators are at variance due to the use of different methods and nomenclature. It is thus apparent that there is a need for further investigations of the fibrinolytic system in the newborn.

This paper is concerned with an investigation of the fibrinolytic system in normal full-term newborns at birth and during the first few days of life.

### CLINICAL MATERIAL AND BLOOD SAMPLING

The clinical material consisted of 57 full-term normal newborns aged 0-5 days. All were born in normal vertex presentation after uncomplicated pregnancy. At 1 and 5 mm old had an Apgar score of 8-9 or 10. Infants with visible jaundice were not accepted.

The clamped cord was cut off under sterile precautions and a plastic catheter (Infant feeding tube French No 8) was introduced into the umbilical cord. Blood was then allowed to run freely into test tubes. This sampling technique was used throughout irrespective of the time after delivery. Between 10-15 ml of blood was obtained from each infant but it

was not always possible to analyse each sample by all methods. Samples were obtained at the earliest 5 mm after delivery. The material was arranged in 10 age groups (Table 2). The oldest infant was 5 days and 20 hours.

In 21 cases from the group 0-30 mm samples were obtained from the placental side of the umbilical cord as well as from the catheterized umbilical vein for comparative studies. Blood from the proximal end of the divided cord was allowed to run directly into test tubes. Milking of the cord was avoided.

Sampling was performed at the department of obstetrics. All samples were transported as quickly as possible to the coagulation laboratory located in the close vicinity in order to get an accurate measure of the fibrinolytic activity. It is important that the time lag between sampling and assay is as short as possible (4, 14, 22, 36). In this study the time lag never exceeded 30 min.

### LABORATORY PROCEDURES

**Collection of blood.** The blood was collected with the silicone technique and citrated plasma and serum were prepared as described previously (39, 43).

The following determinations were made:

1. **Fibrinolytic activity** on unheated and heated fibrin plates according to Anderson *et al.* (4). Fibrinogen from one and the same batch was used for all the assays in this study. The normal ranges on unheated plates for adults at our laboratory are 0-85 mm for plasma and 0-116 mm for the re-suspended erythrocyte precipitate. Mean values 23 mm and 74 mm respectively. There is no activity of plasma on heated plates in healthy adults.

2. **Erythrocyte cation time** according to Nilsson & Olow (40). Normal value for adults is >100 mm.

3. **Fibrinolytic split products** in serum and in plasma from blood collected with the silicone technique. E-aminocaproic acid (EACA) with an immunochemical method described by Näscher (38). In this method an antiserum against the D fraction of the split products is applied to the agarose gel and

- 15 Soupart P. Free amino acids of blood and urine in the human. In J T Holden (ed) *Amino acid pools* Elsevier Amsterdam 1962
- 16 Stokke O, Eldjarn L, Norum K R, Steen Johnsen J & Halvorsen S. Methylmalonic acidemia. A new inborn error of metabolism which may cause fetal acidosis in the neonatal period. *Scand J Clin Lab Invest* 20: 313 1967

Submitted March 14 1969

Accepted June 30 1969

Paediatric Research Institute  
Rikshospitalet  
Oslo  
Norway

**Key words** Methylmalonic acidemia, hyperglycemia, acidosis

5  *$\alpha$ -macroglobulin* according to an esterolytic method by Garrot (25) The result is expressed in per cent and is related to a normal standard consisting of pooled serum from healthy military orderlies about 20 years of age The normal adult range is 80-120

6 *Aminoplasmin* activity so-called progressive anti plasmin according to a modification of Shamaiah & Ramon's caseinolytic method (30) Glycerol activated bovine plasmin (Labs 1375) was added to a 1:20 dilution of the serum and after 120 minutes incubation of the mixture at 37°C the residual plasmin was measured with casein as a substrate for the enzymatic digestion The result is expressed in arbitrary caseinolytic units (ACU) defined in such a way that 10 ACU are equal to the amount of enzyme producing an increase in optical density at 280 m $\mu$  of 0.300 using a 3% casein solution and a digestion time of 20 minutes The normal adult range is 400-600 ACU/ml

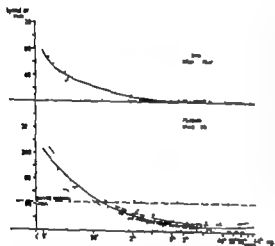


Fig 1 Fibrinolytic activity of plasma on unheated and heated fibrin plates in 161 infants Correlation between lysed area in mm ( $y$ ) and logarithmed time after delivery ( $x$ ) Since it was obvious that the values fitted badly to a straight line second degree polynomial regression was used The square root of  $y$  was chosen to stabilize the variance at different time periods The regression curves for unheated plates can be derived from the formula

$$\sqrt{y} = 27.9 - 11.14x + 1.64x^2$$

The nonlinear shape of this curve is statistically significant (for both regression coefficients is  $p < 0.001$ ) The broken lines represent the 95% confidence interval The regression curve for heated plates can be derived from the formula

$$\sqrt{y} = 13.34 - 7.04x + 0.90x^2$$

The nonlinear shape of this curve is statistically significant (for both regression coefficients is  $p < 0.001$ ) Confidence interval was not calculated because of many zero values

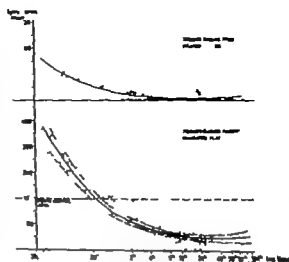


Fig 2 Fibrinolytic activity of resuspended erythrocyte precipitates on unheated and heated fibrin plates in 161 infants Correlation between lysed area in mm ( $y$ ) and logarithmed time after delivery ( $x$ ) Since it was obvious that the values fitted badly to a straight line second degree polynomial regression was used The square root of  $y$  was chosen to stabilize the variance at different time periods The regression curve for unheated plates can be derived from the formula  $\sqrt{y} = 27.45 - 12.67x + 1.88x^2$  The nonlinear shape of this curve is statistically significant (for both regression coefficients is  $p < 0.001$ ) The broken lines represent the 95% confidence interval The regression curve for heated plates can be derived from the formula  $\sqrt{y} = 12.28 - 6.89x + 1.12x^2$  Confidence interval was not calculated since there are many zero values

7 *Inhibitors of the plasminase activation of plasminogen* in serum according to a clot method described by Paraskevas *et al* (43) As pointed out by these authors an extremely high or low content of antiplasmin in the serum will interfere with the method The result is expressed in per cent and is related to a normal standard consisting of pooled serum from 25 healthy military orderlies about 20 years of age The normal adult range is 60-140%

8 *Fibrinogen* according to a modification of Jacobson's method as described by Hubson & Olow (40 a) in which the blood is collected in tubes with and without an inhibitor of fibrinolysis E-aminocaproic acid (EACA) Only the values for blood collected with EACA are given

9 The total serum proteins was measured by the biuret method

10 The venous haematocrit was estimated by the macro method



Table 1 Fibrinolytic activity in umbilical vein blood

Correlations between activity on fibrin plates fibrinolytic split products and euglobulin clot lysis time

Fibrin plates	Split products		Euglobulin clot lysis time
	Serum	Serum EACA	
Plasma	135°		
Unheated plates	$r = +0.65$ $p < 0.001$	—	—
Heated plates	—	137° $r = +0.37$ $p < 0.001$	—
Resusp euglob precipitate	135°		156°
Unheated plates	$r = +0.73$ $p < 0.001$	—	$r = -0.87$ $p < 0.001$
Heated plates	—	137° $r = +0.32$ $p < 0.001$	—

\* No of subjects

the high molecular weight substances (HMWS) serve as standard

4. Plasminogen according to a modification of the immunochemical method by Garrot & Niléhn (27). To avoid activation of plasminogen *in vitro* the blood was collected in tubes containing EACA. We used a specific rabbit antiserum to human plasminogen obtained by immunising rabbits weighing 2.5–3 kg with plasminogen (Grade A freeze dried 25 Spours CU/ampoule from AB Kabi Stockholm). The determination was made according to Laurell (36) with electrophoresis in agarose gel confirming the specific antiserum in dilution 1:200–10 microlitres of each serum sample diluted 1:5 was applied and electrophoresis was allowed to continue for 4 hours at a voltage of 20 V/cm. Mixed serum from 20 healthy volunteers served as reference. The height of the peaks obtained is a measure of the amount of antigen in the samples. Plasminogen and plasmin have the same immunological determinants so that the result of the assay is a measure of the sum of plasminogen and plasmin in the sample.

A comparison was made between determinations of the plasminogen in samples collected in tubes without and with addition of EACA from 20 healthy men and 20 healthy non pregnant women not using oral contraceptives aged 20–50 years. The plasminogen level in the samples with EACA was significantly lower than in samples without EACA ( $p < 0.01$ ). The standard deviation of the plasminogen in the former samples was also smaller (16% and 21% respectively). With the method where EACA was used there was no significant difference between men and women ( $p > 0.1$ ). The range of variation calculated with 2 s.d. in these 40 persons was 61–125% with a mean of 93.

Table 2 Results of determinations of plasminogen inhibitors total serum protein and venous haematocrit in umbilical vein blood

	Range normal adults	Mean	Range	s.d.	No of infants/time after delivery in hours										Correlation to time after delivery	p
					Total	<1/2	1/2–2	2–4	4–8	8–12	12–24	24–36	36–48	48–72	72–96	96–140
Plasminogen antiserum ( )	61–125	43.4	25–130	16.0	162	45	19	25	15	14	23	9	5	5	2	+0.05
α <sub>2</sub> macroglobulin ( )	80–120	109.7	78–291	37.3	161	44	18	23	16	12	25	10	5	5	2	-0.04
Antiplasmin —	400–600	478.9	340–516	79.6	31	19	3	7	2	—	—	—	—	—	—	-0.14
ACU/ml Inhibitor of plasminogen	60–140	183.7	84–490	76.2	148	45	15	17	14	10	24	11	6	4	2	-0.10
Activation ( )	6.6–8.0	7.43	5.9–8.4	0.6	148	36	22	15	16	13	24	11	6	5	2	-0.16
Total serum protein (g/100 ml)	8.43–9.39	8.92	43–62	10.5	66	22	—	—	3	3	17	9	5	5	2	+0.16
Venous haematocrit ( )																0.04
toct ( )																0.2

tubes with EACA contained only small amounts of split products from the very beginning at 0.7 mg/100 ml (mean 1.2 mg/100 ml) and no measurable amounts after 8 hours of age. Statistical analysis of the results is presented in the legend to Fig. 4.

There was a good agreement between the three methods used for measuring fibrinolytic activity. Table 1 shows certain correlations between the activity on fibrin plates, split products and euglobulin clot lysis time. The correlation between the activity of plasma and resuspended euglobulin precipitate, respectively and split products in serum was statistically significant as was the correlation between activities on heated plates and split products in serum obtained in the presence of EACA ( $p < 0.001$ ). The correlation between the activity of resuspended euglobulin precipitate and euglobulin clot lysis time was also statistically significant ( $p < 0.001$ ).

**Plasminogen** (Table 2). The level was on the average 43.4 (range 20–130) of the normal adult value. The correlation with time after delivery was not statistically significant. **Macroglobulin** (Table 2) was moderately higher throughout the groups compared to the normal adult value. The mean was 169.7 with a range of 78–291.

**Inhibitors of plasminogen activation** (Table 2). A higher level than in normal adults was found with a mean of 183.7 (range 84–490).

**Antiplasmin** (Table 2) could be assayed in only 31 cases because of shortage of serum. The results obtained fell within the normal range for adults with a mean of 478.9 ACU/ml (range 340–516 ACU/ml).

The correlation between the values for the different inhibitors in time after delivery was not statistically significant.

**Fibrinogen** (Fig. 5). The mean value for the first 24 hours bordered to the lower normal range for adults (0.26 g/100 ml) with a relatively wide spread (0.13–0.43 g/100 ml). On the second day there was a tendency to higher

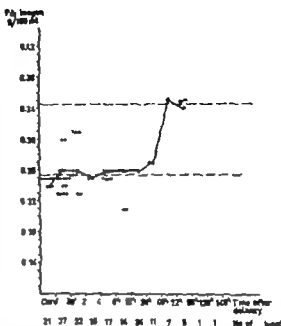


Fig. 5. Fibrinogen values (g/100 ml) in cord blood (21 subjects) and in umbilical vein blood (155 subjects). The unbroken line represents the mean values. There is a significant increase in the mean value on the third day of life ( $p < 0.01$ ). The broken lines represent the adult normal range.

values with a mean of 0.27 g/100 ml and a range of 0.20–0.36 g/100 ml. From the third day on the values were significantly higher ( $p < 0.01$ ) with a mean of 0.35 g/100 ml (range 0.24–0.44 g/100 ml).

**Total serum protein** (Table 2) was checked in 148 cases and agreed with reported normal values for the newborn period (51). The mean value for the whole material was 7.4 g/100 ml (range 5.9–8.4 g/100 ml). There was a small but significant decrease with increasing time after delivery.

**Haematocrit values** (Table 2) were obtained in 66 cases and agreed with those reported by Osler & Nauman (42) but were lower than those of Usher *et al.* (57). The mean value for all cases was 52.4 with a range from 43 to 62. There was no significant correlation in time after delivery but unfortunately no determinations were made between 30 minutes and 4 hours after parturition when the plasma volume is known to decrease (57).

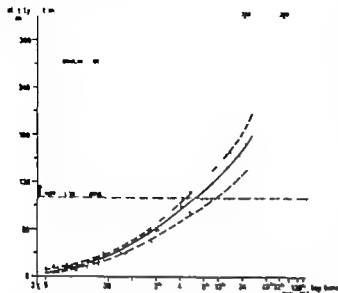


Fig 3 Euglobulin clot lysis times in 155 infants. Correlation between clot lysis time (y) and logarithmed time after delivery (x). Since it was obvious that the values fitted badly to a straight line second degree polynomial regression was used. The regression curve can be derived from the formula  $\log y = 0.06 + 1.13x - 0.14x^2$ . The nonlinear shape of this curve is statistically significant (for both regression coefficients is  $p < 0.001$ ). The broken lines represent the 95% confidence interval.

## RESULTS

### Umbilical vein blood

**Fibrinolytic activity on fibrin plates** The results are given in Figs 1 and 2. In samples obtained within half an hour of parturition both the plasma and the resuspended euglobulin precipitate contained considerable fibrinolytic activity as measured on unheated plates. Values between 31–355 mm (mean 180 mm) were noted for plasma and between 93–425 mm (mean 260 mm) for the resuspended euglobulin precipitate. The activity successively fell, and after 4 hours it was within the normal range for adults.

There was also a plasmin activity as measured on heated plates. In the group where the samples were taken within the first half hour of parturition, the activity was 18–121 mm (mean 50 mm) for plasma and 14–99 mm (mean 50 mm) for the resuspended euglobulin precipitate. The plasmin activity fell rapidly and was no longer demonstrable in samples collected 4 hours after parturition.

In all four measurements of fibrinolytic ac-

tivity (plasma and resuspended euglobulin precipitate on unheated and heated plates) the correlation between lysed area and the time after delivery was statistically significant as noted in the legends to Figs 1 and 2.

**Euglobulin clot lysis time** (Fig 3). In samples obtained within half an hour of delivery the mean value found was 13 minutes. The lysis times then became successively longer and already 4 hours after parturition they fell within the normal range for adults ( $> 100$  min). Statistical analysis of the findings is presented in the legend to Fig 3.

**Fibrinolytic split products** (Fig 4). Serum from sample collected within the first half hour of parturition contained split products 2–70 mg/100 ml with a mean of 16 mg/100 ml. The amounts then decreased with increasing interval between parturition and sampling. From 8–12 hours and on split products, if any were demonstrable in only small amounts ( $< 4$  mg/100 ml).

Serum prepared from blood collected in

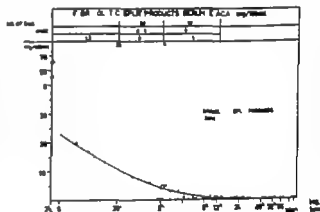


Fig 4 Fibrinolytic split products in serum (174 infants) and serum with EACA (168 infants). Correlation between split products in serum (y) and logarithmed time after delivery (x). Since it was obvious that the values fitted badly to a straight line second degree polynomial regression was used. The regression curve can be derived from the formula  $y = 39.88 - 26.13x + 4.28x^2$ . The nonlinear shape of this curve is statistically significant (for both regression coefficients is  $p < 0.001$ ). Confidence interval was not calculated because of many zero values after about 8 hours. For split products in serum with EACA range and mean values in the different age groups are given. The correlation with time after delivery is statistically significant ( $r = -0.45$ ,  $p < 0.001$ ).

tubes with EACA contained only small amounts of split products from the very beginning i.e. 0-7 mg/100 ml (mean 1.2 mg/100 ml) and no measurable amounts after 8 hours of age. Statistical analysis of the results is presented in the legend to Fig. 4.

There was a good agreement between the three methods used for measuring fibrinolytic activity. Table 1 shows certain correlations between the activity on fibrin plates, split products and euglobulin clot lysis time. The correlation between the activity of plasma and resuspended euglobulin precipitate respectively and split products in serum was statistically significant as was the correlation between activities on heated plates and split products in serum obtained in the presence of EACA ( $p < 0.001$ ). The correlation between the activity of resuspended euglobulin precipitate and euglobulin clot lysis time was also statistically significant ( $p < 0.001$ ).

**Plasminogen** (Table 2). The level was on the average 43.4 (range 25-130) of the normal adult value. The correlation with time after delivery was not statistically significant.  $\alpha_2$  macroglobulin (Table 2) was moderately higher throughout the groups compared to the normal adult value. The mean was 169.7 with a range of 78-291.

**Inhibitors of plasminogen activation** (Table 2). A higher level than in normal adults was found with a mean of 183.7 (range 84-490).

**Anthraxan** (Table 2) could be assayed in only 31 cases because of shortage of serum. The results obtained fell within the normal range for adults with a mean of 478.9 ACU/ml (range 340-516 ACU/ml).

The correlation between the values for the different inhibitors to time after delivery was not statistically significant.

**Fibrinogen** (Fig. 5). The mean value for the first 24 hours bordered to the lower normal range for adults (0.26 g/100 ml) with a relatively wide spread (0.13-0.43 g/100 ml). On the second day there was a tendency to higher

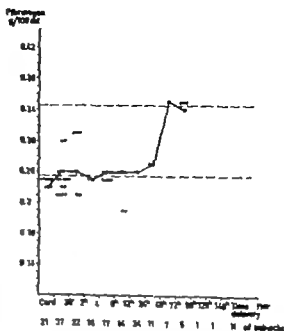


Fig. 5 Fibrinogen values (g/100 ml) in cord blood (21 subjects) and in umbilical vein blood (155 subjects). The unbroken line represents the mean values. There is a significant increase in the mean value on the third day of life ( $p < 0.01$ ). The broken lines represent the adult normal range.

values with a mean of 0.27 g/100 ml and a range of 0.20-0.36 g/100 ml. From the third day on the values were significantly higher ( $p < 0.01$ ) with a mean of 0.35 g/100 ml (range 0.24-0.44 g/100 ml).

**Total serum protein** (Table 2) was checked in 148 cases and agreed with reported normal values for the newborn period (51). The mean value for the whole material was 7.4 g/100 ml (range 5.9-8.4 g/100 ml). There was a small but significant decrease with increasing time after delivery.

**Haematocrit values** (Table 2) were obtained in 66 cases and agreed with those reported by Oski & Naman (42) but were lower than those of Usher *et al.* (57). The mean value for all cases was 52.4 with a range from 43 to 62. There was no significant correlation to time after delivery but unfortunately no determinations were made between 30 minutes and 4 hours after parturition when the plasma volume is known to decrease (57).

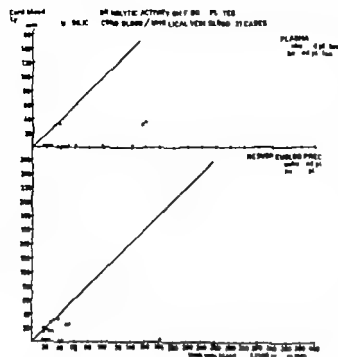


Fig 6 Comparison between fibrinolytic activity on fibrin plates of cord blood and umbilical vein blood 21 cases

### B Comparison between umbilical vein blood and cord blood

In 21 cases a comparison was made between the fibrinolytic activity and all the other above mentioned factors in cord blood and umbilical vein blood obtained 5–22 minutes after parturition. The results are shown in Fig 6 and Table 3.

Fibrinolytic activity on fibrin plates showed a significantly higher ( $p < 0.001$ ) plasminogen

activator and plasmin activity in the umbilical vein blood than in cord blood with a range of 100–355 mm for plasma and 182–373 mm for resuspended euglobulin precipitate. In cord blood the range was 0–146 mm for plasma and 0–249 mm for resuspended euglobulin precipitate.

The euglobulin clot lysis times were also significantly shorter for umbilical vein blood (5–20 min) than for cord blood (10–330 min). The mean difference was 59 min ( $p < 0.001$ ).

The split products in serum from umbilical vein blood were present in significantly larger quantities (8–50 mg/100 ml) than in serum from cord blood (0–30 mg/100 ml). The mean difference was 12.6 mg/100 ml ( $p < 0.01$ ).

In serum obtained in the presence of EACA split products were demonstrated in 7 cases from umbilical vein blood and in 5 cases from cord blood. The highest values were 5 mg/100 ml and 3 mg/100 ml respectively without significant differences between the groups.

As to plasminogen  $\alpha$ -macroglobulin and fibrinogen no significant differences were obtained.

Inhibitors of plasminogen activation were significantly higher in the umbilical vein blood than in cord blood. The mean difference was 38.4 ( $p < 0.05$ ).

Total serum protein and venous haematocrit were significantly higher in the umbilical vein

Table 3 Comparison between umbilical vein blood (V) and cord blood (C)

	No of subjects	V		C		Mean difference between V and C	P
		Range	Mean	Range	Mean		
Plasminogen immunol ( )	14	27–130	59.7	40–103	59.7	$\pm 0$	$> 0.05$
$\alpha_2$ macroglobulin ( )	16	127–253	178.1	74–291	163.5	+14.6	$> 0.05$
Inhibitors of plasminogen activation ( )	18	101–259	180.8	64–258	142.7	+38.1	$< 0.05$
Euglobulin clot lysis time (min)	21	5–20	12	10–330	71	-59	$< 0.001$
Fibrinolytic split products (mg/100 ml)							
(a) serum	19	8–50	31.3	0–30	8.6	+12.7	$< 0.01$
(b) serum — EACA	20	0–5	1.1	0–3	0.5	+0.6	$> 0.05$
Fibrinogen (g/100 ml)	20	0.17–0.37	0.25	0.11–0.37	0.24	+0.01	$> 0.05$
Total serum protein (g/100 ml)	16	6.2–8.3	7.4	5.9–8.0	6.9	+0.5	$< 0.001$
Haematocrit ( )	14	45–59	52	41–60	48.7	+3.3	$< 0.01$

blood than in cord blood ( $p < 0.001$  and  $< 0.01$  respectively) which is also in agreement with earlier investigations already cited above.

## DISCUSSION

Numerous investigations of the fibrinolytic activity in newborns have been published up to date. Most of these studies report results obtained with cord blood (5, 7, 10, 11, 16, 24, 30, 31, 33, 34, 44, 48, 53). Some investigators have studied blood obtained by puncture of the cord vein and/or artery and thereby avoided any admixture of thromboplastic material from umbilical cord tissue (8, 14, 17, 18, 35, 46). Venipuncture of the infant has been used particularly when serial sampling was performed in the first days of life (8, 10, 11, 13, 23, 24, 28, 37). Only in three investigations of term infants (2, 20, 21) has blood been obtained by catheterisation of the umbilical vessels of the infant.

The fibrinolytic activity has been measured by many different methods (e.g. clot lysis of whole blood, plasma or euglobulin fraction, measuring of the fibrinogen after different times of incubation, thromboelastogram, fibrin plates). The results are therefore not strictly comparable. On the whole the fibrinolytic activity in the newborn is reported to be higher than that in normal adults or the mother, but Gabarro *et al.* (24) found lower activity in newborns than in their mothers. Chaplin (14) and Cope & Semmons (18) found the activity in umbilical artery blood to be higher than that in umbilical vein blood. Serial sampling has shown decreasing fibrinolytic activity with increasing interval after delivery (10, 21, 23, 28, 37).

Some investigations (45, 49) have been carried out on serum which, however, gives information only on the plasminogen and inhibitors but not on activator activity.

In the evaluation of the results two very important factors capable of influencing the plasminogen activator activity measured

should be borne in mind, namely the interval between sampling and analysis and the temperature of the sample before analysis. Fearnley (22), Truelove (56), Chaplin (14) and later Nilsson & Olow (40b) stressed that investigation of the activator activity should be performed within half an hour of sampling. Truelove found that cooling of the blood to  $+4^{\circ}\text{C}$  rather increased the activator activity while Nilsson & Olow (40b) showed that freezing reduced it. Berglund (10) who was the first to study the neonatal fibrinolytic activity on fibrin plates (found such activity in only 50% of her series but at the same time pointed out that her results might be falsely low owing to the interval between sampling and analysis). Ambrose *et al.* (2, 3) found only traces of activator activity in healthy full-term newborns but they did not mention the interval between sampling and analysis.

In our investigation which was performed throughout on blood from the catheterised umbilical vein of the infant the fibrinolytic activity was determined in plasma and in the resuspended euglobulin precipitate.

Our results show that all healthy full-term newborns have a plasminogen activator activity which at birth is much higher than that in blood from normal adults. The activity falls to the level of adults within about 4 hours of birth. We also found plasmin activity which may however not be representative of the situation *in vivo* but due to activation of plasminogen *in vitro*. This assumption is strengthened by the fact that fibrinolytic split products were demonstrated mainly in blood obtained without EACA and only in at most small amounts in the blood collected in tubes containing EACA.

A significant correlation was found between the results obtained by the methods used: fibrin plates, euglobulin clot lysis time and determination of the fibrinolytic split products in serum without addition of EACA. The euglobulin clot lysis time is a rather crude measure of the fibrinolytic activity and gives reliable results only when considerable fibrino-

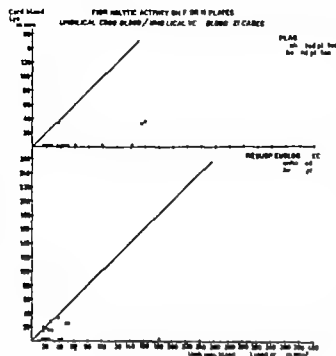


Fig 6 Comparison between fibrinolytic activity on fibrin plates of cord blood and umbilical vein blood 21 cases

### B Comparison between umbilical vein blood and cord blood

In 21 cases a comparison was made between the fibrinolytic activity and all the other above mentioned factors in cord blood and umbilical vein blood obtained 5–22 minutes after parturition. The results are shown in Fig 6 and Table 3.

Fibrinolytic activity on fibrin plates showed a significantly higher ( $p < 0.001$ ) plasminogen

activator and plasmin activity in the umbilical vein blood than in cord blood with a range of 100–355 mm for plasma and 182–373 mm for resuspended euglobulin precipitate. In cord blood the range was 0–146 mm for plasma and 0–249 mm for resuspended euglobulin precipitate.

The euglobulin clot lysis times were also significantly shorter for umbilical vein blood (5–20 min) than for cord blood (10–330 min). The mean difference was 59 min ( $p < 0.001$ ).

The split products in serum from umbilical vein blood were present in significantly larger quantities (8–50 mg/100 ml) than in serum from cord blood (0–30 mg/100 ml). The mean difference was 12.6 mg/100 ml ( $p < 0.01$ ).

In serum obtained in the presence of EACA split products were demonstrated in 7 cases from umbilical vein blood and in 5 cases from cord blood. The highest values were 5 mg/100 ml and 3 mg/100 ml respectively without significant differences between the groups.

As to plasminogen  $\alpha$  macroglobulin and fibrinogen no significant differences were obtained.

Inhibitors of plasminogen activation were significantly higher in the umbilical vein blood than in cord blood. The mean difference was 38.4 ( $p < 0.05$ ).

Total serum protein and venous haematocrit were significantly higher in the umbilical vein

Table 3 Comparison between umbilical vein blood (U) and cord blood (C)

	No. of subjects	U		C		Mean difference between U and C	P
		Range	Mean	Range	Mean		
Plasminogen immunol ( )	14	27–130	59.7	40–103	59.7	$\pm 0$	$> 0.05$
$\alpha_2$ macroglobulin ( )	16	127–253	178.1	74–291	163.5	+14.6	$> 0.05$
Inhibitors of plasminogen activation ( )	18	101–259	180.8	64–258	142.7	+38.1	$< 0.05$
Euglobulin clot lysis time (min)	21	5–20	12	10–330	71	–59	$< 0.001$
Fibrinolytic split products (mg/100 ml)							
(a) serum	19	8–50	21.3	0–30	8.6	+12.7	$< 0.01$
(b) serum — EACA	20	0–5	1.1	0–3	0.5	+0.6	$> 0.05$
Fibrinogen (g/100 ml)	20	0.17–0.37	0.25	0.11–0.37	0.24	+0.01	$> 0.05$
Total serum protein (g/100 ml)	16	6.2–8.3	7.4	5.9–8.0	6.9	+0.5	$< 0.001$
Haematocrit ( )	14	45–59	52	41–60	48.7	+3.3	$< 0.01$

of an inhibitor of fibrinolysis (EACA) which was important because of the high fibrinolytic activity during the first hours after birth. The values therefore reflect the situation *in vivo*. Inhibitors of fibrinolysis have not previously been used in the determination of fibrinogen in the newborn. Reported low values might therefore be due to fibrinogenolysis *in vitro* and normal values might be due to sampling from the cord with lower fibrinolytic activity. But even with the addition of EACA the spread in our series was relatively wide. About 50% of the values in the cord blood and in the vein blood within the first day fell below the lower border of normal adult range (Fig. 5). There was no significant difference between cord blood and umbilical vein blood obtained within 30 min after delivery. At 3 days of age the mean value was significantly higher compared to that for the first 2 days ( $p < 0.01$ ). This difference is in agreement with some earlier studies. Taylor (54) reported an insignificant rise in the mean concentration of fibrinogen during the first several days of life. Fisher *et al.* (23) found the difference to be significant with values in the lower normal range in the first day and definitely normal levels on the 4th day. Recently Hanzlky *et al.* (32) have reported on immunological fibrinogen assays in healthy premature repeatedly studied during the first ten days. They found higher levels already at 4 hours of age and then successively increased values the following days. They conclude that this increase can not be due solely to changes in plasma volume but must be the result of increasing synthesis of fibrinogen. Usher *et al.* (57) have shown that the important shifts in plasma volume of the newborn occur within the first 24 hours with a minimum plasma volume at 4 hours. At 24 hours the plasma volume has stabilised and no significant changes occur during the following days. An increase in fibrinogen concentration at 4 hours might in part be due to the plasma concentration and/or a diminished fibrinogenolysis at that time. Higher values after the first day however can be explained

neither by alterations in plasma volume nor in fibrinolytic activity but speak in favour of an accelerated synthesis.

The fibrinolytic activity develops already during intra uterine life (17, 19, 58) and probably serves to maintain a free foeto-placental circulation (17). The actual stress of delivery may also increase this activity which afterwards rapidly decreases. The question remains whether the increased fibrinolytic activity in the newborn serves any physiological purpose. Its function might be to prevent deposition of fibrin in the alveoli as was first proposed by Phillips & Schroeder (44). A disturbance of the fibrinolytic system has been regarded as one factor in the complex pathogenesis of the development of hyaline membranes in premature (2). Pulmonary ischaemia has been suggested as another important factor (15). In the light of Astrup's observation (6) that the content of the plasminogen activator activity in a tissue is related to its vascularisation and is thus high in well vascularised organs one might imagine that a decreased circulating activator activity in the ischaemic lungs in premature is a contributory cause of the development of hyaline membranes.

## SUMMARY

The fibrinolytic system was studied in 207 normal full-term newborns during the first 5 days of life. A high but rapidly decreasing plasminogen activator activity was found during the first 4 hours in umbilical vein blood in all of the children studied. The activity in the cord blood was lower and cannot be regarded as representative of the child. Fibrinolytic split products were demonstrated in large and rapidly decreasing amounts in serum samples without the addition of EACA but in at most small amounts in serum when EACA was added at sampling. These findings confirm the high plasminogen activator activity at birth which rapidly falls and furthermore show that there are no significant amounts of split products *in vivo* in the newborn. The plasminogen



lysis is present e.g. when the lysis time is less than 100 minutes

The activity of the cord blood was low compared to that of umbilical vein blood, or even absent. This difference also reported by Markarian *et al* (37) probably explains why earlier investigations on umbilical cord blood could not always demonstrate fibrinolytic activity. Sampling of blood from the cut cord or puncture of the umbilical cord vein have given the same results. This makes it less likely that some possible inhibitory substance in Wharton's jelly might lower the fibrinolytic activity in cord blood by contamination.

The difference found in activity between the cord blood and umbilical vein blood can probably be explained by production of activator by the newborn. Many data discussed by Beller *et al* (8) and Markarian *et al* (37) strengthen this assumption. At delivery the mother has low fibrinolytic activity (8) the placenta tissue at term is fibrinolytically inactive (1, 9) and the activity is higher in umbilical artery blood than in vein blood (14, 18). The stress produced by the delivery may also possibly increase the activator activity.

Fibrinolytic split products in cord blood have earlier been demonstrated by Snehm (52) Bonifaci *et al* (12) and Huthaway *et al* (29) by use of precipitin tube technique or immunoelectrophoresis and with an antiserum to fibrinogen. As far as we can judge these authors did not add inhibitors of fibrinolysis when collecting the blood samples. The advantage of the method used by us has been discussed by Nihlen (38).

The findings of large and rapidly decreasing amounts of split products in serum samples without the addition of EACA and only small amounts of split products in serum when EACA was added at sampling speak clearly in favour of a high plasminogen activator activity at birth which rapidly falls. Our opinion on this point is at variance with that of Bonifaci *et al* (12).

Our determination of plasminogen by the immunochemical method where blood is col-

lected in the presence of EACA in order to prevent plasminogen activation in vitro has not previously been used in newborns. We found the values to be about 50% of the normal adult level. This is in agreement with earlier investigations with other methods (2, 3, 8, 13, 17, 41, 44, 45, 49, 55). Our results suggest that the plasminogen level is due to a low content of plasminogen in the infant and not to increased activation to plasmin after sampling. At variance with Beller *et al* (8) we found no significant difference between the plasminogen content of cord blood and umbilical vein blood.

No unanimity has been achieved regarding the inhibitors of fibrinolysis in the newborn. Many earlier investigations report lower values than in adults (35, 45, 47, 48, 49) or in the mother (44) while others have found normal (2, 8) or higher levels (13, 54) than in adults. Apart from Beller *et al* (8) previous workers have not been able to distinguish between inhibitors of plasminogen activation and antiplasmin because in their methods they used plasmin which had been activated by streptokinase or urokinase.

We determined the inhibitors of plasminogen activation  $\alpha$ -macroglobulin and progressive antiplasmin. Inhibitors of plasminogen activation and  $\alpha$ -macroglobulin were moderately higher than the normal adult value. Ganrot & Scherstén (26) have already shown that  $\alpha$ -macroglobulin is increased in umbilical cord blood. Using Ganrot's method we found the same values in the umbilical vein blood.

The antiplasmin method used excludes inhibitors of plasminogen activation because the plasmin used was activated with glycerol and not with streptokinase or urokinase. We found the values to be within the range for adults. The newborn was thus found to have a higher total inhibitor level than normal adults. The high activator activity cannot be ascribed to a low inhibitor content. The function of the high inhibitor level may presumably be to counterbalance the high activator activity.

Fibrinogen was determined in the presence

of an inhibitor of fibrinolysis (EACA) which was important because of the high fibrinolytic activity during the first hours after birth. The values therefore reflect the situation *in vivo*. Inhibitors of fibrinolysis have not previously been used in the determination of fibrinogen in the newborn. Reported low values might therefore be due to fibrinogenolysis *in vitro* and normal values might be due to sampling from the cord with lower fibrinolytic activity. But even with the addition of EACA the spread in our series was relatively wide. About 50% of the values in the cord blood and in the ven blood within the first day fell below the lower border of normal adult range (Fig. 5). There was no significant difference between cord blood and umbilical ven blood obtained within 30 min after delivery. At 3 days of age the mean value was significantly higher compared to that for the first 2 days ( $p < 0.01$ ). This difference is in agreement with some earlier studies. Taylor (54) reported an insignificant rise in the mean concentration of fibrinogen during the first several days of life. Friher *et al* (23) found the difference to be significant with values in the lower normal range in the first day and definitely normal levels on the 4th day. Recently Kantzky *et al* (32) have reported on immunological fibrinogen assays in healthy premature repeatedly studied during the first ten days. They found higher levels already at 4 hours of age and then successively increased values the following days. They conclude that this increase can not be due solely to changes in plasma volume but must be the result of increasing synthesis of fibrinogen. Usher *et al* (57) have shown that the important shifts in plasma volume of the newborn occur within the first 24 hours with a minimum plasma volume at 4 hours. At 24 hours the plasma volume has stabilised and no significant changes occur during the following days. An increase in fibrinogen concentration at 4 hours might in part be due to the plasma concentration and/or a diminished fibrinogenolysis at that time. Higher values after the first day, however, can be explained

neither by alterations in plasma volume nor in fibrinolytic activity but speak in favour of an accelerated synthesis.

The fibrinolytic activity develops already during intra uterine life (17, 19, 58) and probably serves to maintain a free foeto-placental circulation (17). The actual stress of delivery may also increase this activity which afterwards rapidly decreases. The question remains whether the increased fibrinolytic activity in the newborn serves any physiological purpose. Its function might be to prevent deposition of fibrin in the alveoli as was first proposed by Phillips & Schroedel (44). A disturbance of the fibrinolytic system has been regarded as one factor in the complex pathogenesis of the development of hyaline membranes in premature (2). Pulmonary ischaemia has been suggested as another important factor (15). In the light of Astrup's observation (6) that the content of the plasminogen activator activity in a tissue is related to its vascularisation and is thus high in well vascularised organs, one might imagine that a decreased circulating activator activity in the ischaemic lungs in premature is a contributory cause of the development of hyaline membranes.

## SUMMARY

The fibrinolytic system was studied in 207 normal fullterm newborns during the first 5 days of life. A high but rapidly decreasing plasminogen activator activity was found during the first 4 hours in umbilical ven blood in all of the children studied. The activity in the cord blood was lower and cannot be regarded as representative of the child. Fibrinolytic split products were demonstrated in large and rapidly decreasing amounts in serum samples without the addition of EACA but in at most small amounts in serum when EACA was added at sampling. These findings confirm the high plasminogen activator activity at birth which rapidly falls and furthermore show that there are no significant amounts of split products *in vivo* in the newborn. The plasminogen

level was decreased in accordance with previous investigations. The high activator activity is counterbalanced by adequate inhibitory protection with an increase of the  $\alpha$  macroglobulin and inhibitors of plasminogen activation and the adult content of antipain. The mean fibrinogen values in the first 48 hours of life are in the lower part of the normal range but increase on the third day to a significantly higher level.

# ACKNOWLEDGEMENT

The statistical analysis was carried out by Peter Vorwerk, F.K. Institute of Mathematical Statistics, University of Lund, with the computer CD 3600 at Uppsala.

This investigation was supported by grants from the Swedish Medical Research Council (B69-40X 678 04B) and from the Medical Faculty, University of Lund.

# REFERENCES

- 1 Albrechtsen O & Fibrinolytic activity in the organism. *Acta Physiol Scand* 47 Suppl. 165 1959
- 2 Ambrus C M, Weintraub D H, Dunphy D, Dowd J E, Pickren J W, Niswander K R & Ambrus J L. Studies on hyaline membrane disease. I. The fibrinolysis system in pathogenesis and therapy. *Pediatrics* 32 10 1963
- 3 Ambrus C M, Weintraub D H, Niswander K R & Ambrus J L. Studies on hyaline membrane disease. II. The ontogeny of the fibrinolysis system. *Pediatrics* 35 91 1965
- 4 Andersson L, Nilsson I M & Olow H. Fibrinolytic activity in man during surgery. *Thrombosis Diathesis Haemorrh* 7 391 1962
- 5 Arustowicz Z. Euglobulin fibrinolysis in newborn infants: healthy premature and premature with respiratory distress syndrome (RDS). *Ann Paediat* 206 265 1966
- 6 Astrup T. Tissue activators of plasminogen. *Fed Proc* 25 42 1966
- 7 Beller F K. Die Gernnnungsverhältnisse bei der Schwangeren und beim Neugeborenen. Barth Leipzig 1957
- 8 Beller F K, Douglas G W & Epstein M D. The fibrinolytic enzyme system in the newborn. *Am J Obstet Gynec* 96 977 1966
- 9 Beller F K, Goessens W & Herrschlein H J. Tissue activator of the fibrinolytic system in placental tissue. *Obstet Gynecol* 20 117 1962
- 10 Berglund G. The fibrinolytic activity in the newborn. *Acta Paediat Scand* 47 511 1958

- 11 Bernardi M, Grasso E, Nasso S, Verga A & Albanesi R. Sulla patogenesi delle emorragie del neonato. *Il Lattante* 24 219 1953
- 12 Bonifazi E, Baggio P & Gravina E. Demonstration of split products of fibrinogen in the blood of normal newborns. *Biol Neonat* 12 29 1968
- 13 Bruster H & Pfitzner W. Untersuchungen über das fibrinolytische Potential bei reifgeborenen und jungen Säuglingen. *Mischr Kinderheilk* 113 476 1965
- 14 Chaplin H. Observations on fibrinolysis in umbilical cord blood. *Clin Sci* 13 111 1954
- 15 Chu J, Clements J A, Cotton E K, Klaus M H, Sweet A Y & Tooley W H. Neonatal pulmonary ischemia. Part I. Clinical and physiological studies. *Pediatrics* 40 709 1967
- 16 Ciulla U & Lurisch C. Lattenza fibrinolitica del siero di sangue del feto. *Ann Obster Gynec* 76 207 1954
- 17 Cope I & Mitchell P. The plasminogen-plasmin content of umbilical cord blood. *Aust N Z J Obstet Gynec* 4 117 1964
- 18 Cope I & Simmons H. The fibrinolytic activity of umbilical cord blood. *J Obstet Gynecol Brit Emp* 65 416 1958
- 19 Ekelund H, Hedner U & Åstedt B. Fibrinolysis in the foetus. To be published
- 20 Engstrom L & Lager L. Changes in plasma fibrinolytic activity of newborn infants during first hour after birth. *Acta Paediat Scand* 53 326 1964
- 21 —. Fibrinolytic activity in plasma of newborn infants. *Acta Paediat Scand* 53 379 1964
- 22 Fearnley G R, Revell R & Moore Tweed J. Observations on the inactivation of fibrinolytic activity in shed blood. *Clin Sci* 11 309 1952
- 23 Fisher S, Schwartz M, Gottlieb A, Ben Yoseph N & Shapiro S. Fibrinolysis, fibrinogen and factor XIII in newborn infants. *Thrombosis Diathesis Haemorrh* 20 547 1968
- 24 Garburo D, Panuzzi G F & Bonifazi E. Les alterations du système fibrinolytique chez le nouveau né. *Ann de Pédiatrie* 38 621 1962
- 25 Grinrot P O. Determination of  $\alpha$ -macroglobulin as trypsin protein esterase. *Clin Chim Acta* 14 493 1966
- 26 Grinrot P O & Scherstén B. Serum  $\alpha$ -macroglobulin concentration and its variation with age and sex. *Clin Chim Acta* 15 113 1967
- 27 Grinrot P O & Nilén J E. Immunochemical determination of human plasminogen. *Clin Chim Acta* 22 335 1968
- 28 Grossman B J, Heyn R M & Rozenfeld I H. Coagulation studies in the newborn infant. I. Normal infants. *Pediatrics* 9 182 1952
- 29 Hathaway W E, Mull M M & Peebet G S. Disseminated intravascular coagulation in the newborn. *Pediatrics* 43 233 1969
- 30 Howell W L. Fibrinolytic activity of the umbilical cord blood. *Med Ann D C* 29 491 1960

- 31 Jenny J & Duckert F Die Gerinnungsverhältnisse beim lebensfrühen nichtasphyktischen Neugeborenen nach normaler Geburt und Schwangerschaft *Gynaecologia* 155 175 1963
- 32 Kantaky D, Pringheim W, Ziemer O & Kunzer W Veränderungen der Fibrinogenkonzentration im Plasma gesunder Fruchtborenen am 1. Lebensstag *Z Kinderheilk* 105 73 1969
- 33 Kunzer W & Krause E Zur Fibrinogenolyse im Nabelvenen- und Erwachsenenblut *Klin Wochschr* 41 1085 1963
- 34 Kunzer W & Mariel A Der Proaktivator des fibrinolytischen Systems im Nabelvenen- und Sauglingsblut *Ann Paediatr* 202 278 1964
- 35 Larnes, M J, Soulier J P & Mushinski A Le sang du cordon ombilical *Etudes Néonatales* 1 1 1952
- 36 Lattrell C B Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies *Anal Biochem* 15 45 1966
- 37 Marikawa M, Gubens J H, Jackson J J, Benson A F, Landry A, Rosenblatt E, Martorell R & Lobchenco L O Fibrinolytic activity in premature infants: Relationship of the enzyme system to the respiratory distress syndrome *Am J Dis Child* 113 31 1967
- 38 Naftha J E Separation and estimation of split products of fibrinogen and fibrin in human serum *Thrombos Diathes Haemorrh* 18 3 1967
- 39 Nilsson I M, Blomback M & von Praetere, I On an inherited autosomal haemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand* 159 35 1957
- 40 a Nilsson I M & Olsson B Determination of fibrinogen and fibrinogenolytic activity *Thrombos Diathes Haemorrh* 8 297 1962
- 40 b — Fibrinolyses induced by streptokinase in man *Acta Chir Scand* 123 247 1962
- 41 Oetzel J Über das Altersabhängigkeit von Retraction und Profibrinolyse *Anaesthet d. Prax* 77 487 1959
- 42 O'Leary A & Naxson J L Hematologic problems in the newborn Saunders Philadelphia and London 1966 p 10
- 43 Parakevas M, Nilsson I M & Martinsson O A method for determining serum inhibitors of plasminogen activation *Scand J Clin Lab Invest* 14 135 1966
- 44 Phillips L L & Skrodets V A comparison of the fibrinolytic enzyme system in maternal and umbilical-cord blood *Pediatrics* 22 715 1958
- 45 Que P G & Wannemaker L W The plasminogen-plasmin system of newborn infants *Am J Dis Child* 100 836 1960
- 46 Ruge H, Hartert I & Escher W Vergleichende Untersuchungen der Blutgerinnung im mütterlichen und kindlichen Blut bei noch pulsierender Nabelschnur *Gynaecologia* 138 337 1954
- 47 Salazar de Sousa C Les alterations de la coagulabilité sanguine chez le nouveau né *Pédiatrie* 9 787 1954
- 48 — Clotting factors in newborn *Etudes Néonatales* 5 97 1956
- 49 Sammaris E A & Cook C D The relationship between age and fibrinolytic activity of serum *Acta Paediatr Scand* 49 724 1960
- 50 Shimashi Y & Rydon A The plasma inhibitors of plasmin A method for their estimation *Thrombos Diathes Haemorrh* 12 119 1964
- 51 Smith C A The physiology of the newborn infant Thomas Springfield Ill 1959 3rd ed p 266
- 52 Stehan E R Split products of fibrinogen in cord serum *Abstr Paediat Res* 1 221 1967
- 53 Stroder J & Kunzer W Gerinnungsstudien bei Kindern 4 Mitteilung Fibrinogen und Fibrinolyse im Nabelschnurblut *Acta Paediatr* 188 207 1957
- 54 Taylor P M Concentration of fibrinogen in the plasma of normal newborn infants *Pediatrics* 19 233 1957
- 55 Townsend P M The determination of factors in fibrinolysis in umbilical venous blood *Acta Paediatr* 192 155 1959
- 56 Truelove S C The lability of human fibrinolysin *Chin Sci* 12 75 1953
- 57 Usher R, Shephard M & Lind J The blood volume of the newborn infant and placental transfusion *Acta Paediatr Scand* 52 497 1963
- 58 Zillmer H, Ottlein A M & Mattson T Blood clotting and fibrinolysis in human foetuses *Boel Neonat* 10 108 1966

Submitted June 18 1969

Accepted July 16 1969

(H E) Dept of  
Paediatrics  
General Hospital  
214 01 Malmö  
Sweden

**Key words** Fibrinolysis plasminogen inhibitors of fibrinolysis fibrinolytic split products fibrinogen newborn

level was decreased in accordance with previous investigations. The high activator activity is counterbalanced by adequate inhibitory protection with an increase of the  $\alpha$  macro globulin and inhibitors of plasminogen activation and the adult content of antiplasmin. The mean fibrinogen values in the first 48 hours of life are in the lower part of the normal range but increase on the third day to a significantly higher level.

### ACKNOWLEDGEMENT

The statistical analysis was carried out by Peter Vorwerk, F.R., Institute of Mathematical Statistics, University of Lund, with the computer CD 3600 at Uppsala.

This investigation was supported by grants from the Swedish Medical Research Council (B69-40X 678 04B) and from the Medical Faculty, University of Lund.

### REFERENCES

- Albrechtsen O A. Fibrinolytic activity in the organism. *Acta Physiol Scand* 47 Suppl 165 1959.
- Ambrus C M, Weintraub D H, Dunphy E, Dowd J E, Pickren J W, Niswander K R & Ambrus J L. Studies on hyaline membrane disease I. The fibrinolysis system in pathogenesis and therapy. *Pediatrics* 32 10 1963.
- Ambrus C M, Weintraub D H, Niswander K R & Ambrus J L. Studies on hyaline membrane disease II. The ontogeny of the fibrinolysis system. *Pediatrics* 35 91 1965.
- Andersson L, Nilsson I M & Olow B. Fibrinolytic activity in man during surgery. *Thromb Diathes Haemorrh* 7 391 1962.
- Arustowicz Z. Euglobulin fibrinolysis in newborn infants: healthy pretermures and pretermures with respiratory distress syndrome (RDS). *Ann Paediat* 206 265 1966.
- Astrup T. Tissue activators of plasminogen. *Fed Proc* 25 42 1966.
- Beller F K. Die Gerinnungsverhältnisse bei der Schwangerschaft und beim Neugeborenen. Barth, Leipzig 1957.
- Beller F K, Douglas G W & Epstein M D. The fibrinolytic enzyme system in the newborn. *Am J Obstet Gynec* 96 977 1966.
- Beller F K, Goessner W & Herrachlein H J. Tissue activator of the fibrinolytic system in placental tissue. *Obstet Gynecol* 20 117 1962.
- Berglund G. The fibrinolytic activity in the newborn. *Acta Paediat Scand* 47 511 1958.
- Bernardi M, Grasso E, Nasso S, Verga A & Albanesi R. Sulla patogenesi dello emorragie del neonato. *Il Lattante* 24 219 1953.
- Bonifazi E, Baggio P & Gravina E. Demonstration of split products of fibrinogen in the blood of normal newborns. *Biol Neonat* 12 29 1968.
- Bruster H & Pfitzner W. Untersuchungen über das fibrinolytische Potential bei reifgeborenen und jungen Säuglingen. *Mischr Kinderheilk* 113 476 1965.
- Chaplin H. Observations on fibrinolysis in umbilical cord blood. *Clin Sci* 13 111 1954.
- Chu J, Clements J A, Cotton E A, Klaus M H, Sweet A Y & Tooley W H. Neonatal pulmonary ischemia. Part I. Clinical and physiological studies. *Pediatrics* 40 709 1967.
- Cuiffr U & Luraschi C. Lattività fibrinolitica del siero di sangue del fucicolo ombelicale. *Ann Obstet Gynec* 76 207 1954.
- Cope I & Mitchell P. The plasminogen-plasmin content of umbilical cord blood. *Aust N Z J Obstet Gynec* 4 117 1964.
- Cope I & Summons E. The fibrinolytic activity of umbilical cord blood. *J Obstet Gynec Brit Emp* 65 416 1958.
- Ekelund H, Hedner U & Åstedt B. Fibrinolysis in the foetus. To be published.
- Engström L & Lager L. Changes in plasma fibrinolytic activity of newborn infants during first hour after birth. *Acta Paediat Scand* 53 326 1964.
- Fibrinolytic activity in plasma of newborn infants. *Acta Paediat Scand* 53 329 1964.
- Fearnley G R, Revell R & Moore Tweed J. Observations on the inactivation of fibrinolytic activity in shed blood. *Clin Sci* 11 309 1952.
- Fisher S, Schwartz M, Gottlieb A, Ben Yoseph N & Shripuro S. Fibrinolysis, fibrinogen and factor XIII in newborn infants. *Thromb Diathes Haemorrh* 20 542 1968.
- Gaburro D, Panizza G F & Bonifazi E. Les alterations du système fibrinolytique chez le nouveau né. *Ann de Pédiatrie* 38 621 1962.
- Ganrot P O. Determination of  $\alpha$ -macroglobulin as trypsin protein esterase. *Clin Chim Acta* 14 493 1966.
- Ganrot P O & Schersten H. Serum  $\alpha$ -macroglobulin concentration and its variation with age and sex. *Clin Chim Acta* 15 113 1967.
- Ganrot P O & Nilén J E. Immunochemical determination of human plasminogen. *Clin Chim Acta* 22 335 1968.
- Grossman B J, Heyn R M & Rosenfeld I H. Coagulation studies in the newborn infant. I. Normal infants. *Pediatrics* 9 182 1952.
- Hathaway W E, Mutt M M & Pechet G E. Disminated intravascular coagulation in the newborn. *Pediatrics* 43 233 1969.
- Howell W L. Fibrinolytic activity of the umbilical cord blood. *Med Ann D C* 29 491 1960.

- 31 Jenny I & Duckert P Die Gerinnungsverhalten beim lebensfrühen, nichtseptischen Neugeborenen nach normaler Geburt und Schwangerschaft *Gynaecologia* 155 125 1963
- 32 Kuntzky D Fringshagen W Zimmer O & Kunzer W Veränderungen der Fibrinogenkonzentration im Plasma gesunder Frühgeborener am 1. Lebensstag *Z Kinderheilk* 105 73 1969
- 33 Kunzer W & Krume E Zur Fibrinogenolyse im Nabelvein und Erwachsenenblut. *Klin Wochschr* 41 1085 1963
- 34 Kunzer W & Merkel A Der Proaktivator des fibrinolytischen Systems im Nabelvein und Sauglingsblut *Ann Paediat* 202 278 1964
- 35 Larnier M J Souther J P & Binkowski A Le sang du cordon ombilical *Etudes Neonatales* 1 1 195
- 36 Laurel C B Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies *Anal Biochem* 15 45 1966
- 37 Markarian M Oshers J H Jackson J J Bannion A E Lindley A Rosenblatt E, Marzorelli R & Lubchenco L O Fibrinolytic activity in premature infants: Relationship of the enzyme system to the respiratory distress syndrome *Am J Dis Child* 113 312 1967
- 38 Nidehi J E Separation and estimation of split products of fibrinogen and fibrin in human serum *Thrombosis Diathesis Haemorrh* 18 3 1967
- 39 Nilsson I M, Blombäck M & von Francken J On an inherited autosomal haemorrhagic diathesis with antithrombotic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand* 199 35 1957
- 40 a Nilsson I M & Olow B Determination of fibrinogen and fibrinogenolytic activity *Thrombosis Diathesis Haemorrh* 8 297 1962
- 40 b — Fibrinolysis induced by streptokinase in man *Acta Clin Scand* 123 247 1967
- 41 Oshers J Über die Abhängigkeit von Retraction und Profibrinolysin. *Kinderarzt Praxis* 27 482 1959
- 42 Orski F A & Naxos J L *Hematologic problems in the newborn* Saunders Philadelphia and London 1966 p 10
- 43 Parakevas M Nilsson I M & Martinsson G A method for determining serum inhibitors of plasminogen activation *Scand J Clin Lab Invest* 14 138 1962
- 44 Phillips L L & Skrodels V A comparison of the fibrinolytic enzyme system in maternal and umbilical-cord blood *Pediatrics* 27 715 1958
- 45 Qwe H G & Wannemaker L W The plasminogen-plasmin system of newborn infants *Am J Dis Child* 100 836 1960
- 46 Ruge H Hartert I & Eicher W Vergleichende Untersuchungen der Blutgerinnung im mat. fetischen und kindlichen Blut bei noch pulsierender Nabelschnur *Gynaecologia* 138 337 1954
- 47 Salazar de Sousa C Les alterations de la coagulabilité sanguine chez le nouveau né *Pediatrics* 9 787 1954
- 48 — Clotting factors in newborn *Etudes Neonatales* 5 97 1956
- 49 Samartins E A & Cook C H The relationship between age and fibrinolytic activity of serum *Acta Paediat Scand* 49 724 1960
- 50 Shemash Y & Rimon A The plasma inhibitors of plasmin. A method for their estimation. *Thrombosis Diathesis Haemorrh* 12 119 1964
- 51 Smith C A *The physiology of the newborn infant* Thomas, Springfield Ill 1959 3rd ed p 266
- 52 Stueben E R Split products of fibrinogen in cord serum *JAMA/Pediat Res* 1 221 1967
- 53 Stroder I & Kunzer W Gerinnungsstörungen bei Kindern 4. Mitteilung Fibrinogen und Fibrinolyse im Nabelschnurblut. *Ann Paediat* 188 207 1957
- 54 Taylor P M Concentration of fibrinogen in the plasma of normal newborn infants *Pediatrics* 19 233 1957
- 55 Townsend P M The determination of factors in fibrinolysis in umbilical venous blood *Ann Paediat* 192 15 C 1959
- 56 Trudlove S C The lability of human fibrinolysin *Clin Sci* 12 75 1953
- 57 Usher R, Shepherd H & Lind J The blood volume of the newborn infant and placental transfusion. *Acta Paediat Scand* 52 497 1963
- 58 Ziliacov H, Orskov A M & Mattson T Blood clotting and fibrinolysis in human foetuses *Biol Neonat* 10 108 1966

Submitted June 18 1969

Accepted July 16 1969

(H E.) Dept of  
Paediatrics  
General Hospital  
214 01 Malmö  
Sweden

**Key words:** Fibrinolysis, plasminogen, inhibitors of fibrinolysis fibrinolytic split products fibrinogen, newborn

## THE RESULTS OF IMMUNOSUPPRESSIVE THERAPY IN CHRONIC RENAL DISEASES IN CHILDREN AND THE ASSESSMENT OF ERYTHROBLASTS BEHAVIOUR

B HALIKOWSKI, A KUCHARSKA, S GARWICZ, J WYSZAŃSKI and  
K SANCEWICZ PACH

*From the second Paediatric Clinic (Head B Halikowski) Paediatric Institute  
of the Medical Academy in Cracow, Cracow, Poland*

Immunosuppressive therapy (1, 5, 11, 13, 14, 17, 18) has been employed in forms of chronic renal diseases in children in which other therapeutic methods including corticosteroids had proved ineffective.

### MATERIAL AND METHODS

6 Mercaptopurine was used in doses of 2.5 mg/kg/48 hr for periods of 3 weeks. This therapy was usually associated with 0.5 mg/kg/24 hr of Encorton.

Immunosuppressive therapy was administered to 33 children aged 2-17 years (18 boys and 15 girls). There were 9 cases of primary nephrotic syndrome, 8 cases in the course of chronic glomerulonephritis, 12 with chronic glomerulonephritis and 4 with pyelonephritis. The diagnosis was established mainly on the basis of clinical criteria and on long term supervision. In no case was renal biopsy performed.

Renal and liver function tests and peripheral blood counts were carried out in all the children before the beginning of therapy and 1-3 days after it finished.

In 18 children aspiration myelograms and careful repeated examinations of peripheral blood were done. Nine of these 18 had primary or secondary nephrotic syndrome (group A) and nine had chronic glomerulo- or pyelonephritis (group B).

Effectiveness of the therapy was assessed by a scoring system assessing the regression of the symptoms and signs characteristic of each group. Improvement scored 80-100% was regarded as complete remission, 40-70% as partial remission and cases with scores of less than 40% were considered unimproved.

In primary nephrotic syndrome the total protein level, albumin,  $\alpha$ -globulin and cholesterol in the plasma were measured as was proteinuria. Return of the above parameters to their normal values was

scored 20, partial normalization 10 and no effect 0. The sum of scores represented the percentage of complete remission.

For example if a complete normalization of protein plasma level, albumin and  $\alpha$ -globulin was obtained—the score was 60. Partial normalization of cholesterol was scored at 10 points and reduction of proteinuria another 10 points. In such a case the percentage of complete remission after therapy was 80. In the secondary nephrotic syndrome erythrocyturia was used instead of  $\alpha$ -globulin.

In chronic glomerulonephritis the degree of erythrocyturia in relation to therapy was assessed. Complete disappearance of erythrocyturia was taken as 100%. 90 stood for the persistence of microhematuria (less than 10 million of erythrocytes in 12 hours urine), 60 indicated the reducing of macrohematuria to about one half of the initial number (macrohematuria means more than 20 million of erythrocytes in the urine), 0 signified lack of effect.

In pyelonephritis the leucocytes, erythrocytes and bacteria counts were carried out but no effect after immunosuppressive therapy was noticed.

### RESULTS

Using this scoring system the summary review of the effects of immunosuppressive therapy in the presented clinical material is shown in Table 1.

Out of 9 children with primary nephrotic syndrome only two did not improve during the period of immunosuppressive therapy.

Of these two a girl K.E. aged 3 years had been born from a third pregnancy during which the mother had been treated with Dara-

Table 1 *Collective results of treatment*

Diagnosis	No of cases	No effect	Partial remission	Complete remission
Primary nephrotic syndrome	9	2	1	6
Secondary nephrotic syndrome	8	2	5	1
Glomerulonephritis	12	1	5	6
Pyelonephritis	4	3	—	1
Total	33	8	11	14
Percentage	100	24.3	33.3	42.4

prim because of toxoplasmosis. Two younger children had died immediately after birth. Illness had begun a year earlier and despite hospital treatment remission was not achieved. During the child's clinical stay her condition continuously deteriorated. She died after having varicella and pneumonia.

A boy S.W. 2 years was resistant to corticosteroids from the beginning of the illness. Accompanying pneumonia influenced prognosis adversely. Autopsy revealed membranous glomerulonephritis and pneumocystosis.

As can be seen in Table 1 the results obtained in the secondary nephrotic syndrome were less impressive. It is worth noting on the other hand that the remissions persisted and in two children further improvement was observed without additional therapy. In three children with partial remissions 6 MP therapy was repeated resulting in a complete remission in one case.

In the group of children with chronic glomerulonephritis complete or partial remission were achieved in all but one case a boy

Table 2 *The effect of immunosuppressive therapy on bone marrow results of treatment and toxic manifestations*

Patient (initials)	Age (years)	Sex	Erythroblasts		G/E ratio		Results of treatment			Toxic manifestations			
			Before treat- ment	After treatment	Before treatment	After treatment	No effect	Part remission	Complete remission	None	Blood	Liver	
Group A													
K. A.	3		17.7	10.8	3.33	4.27							
D. T.	11	♂	6.6	12.9	9.31	3.81			+		+		
V. B.	7		5.8	16.4	12.00	2.60			+		+		
R. J.	9	♂	11.2	7.0	5.32	8.55			+		+		
N. M.	4	♀	7.5	18.0	8.44	2.66			+		+		
S. W.	13	♂	5.4	18.3	11.83	3.06			+		+		
W. B.	7		11.1	17.5	5.43	3.82		+					
D. H.	9	♂	3.0	12.9	25.00	5.14		+				+	
P. A.	16		7.8	16.5	6.46	3.34	+				+	+	
Arithmetic mean			8.4	13.9	9.68	4.13	Total	1	2	6	7	1	2
Group B													
N. R.	5	♂	25.3	16.4	1.75	3.09							
W. Z.	10		25.8	19.5	1.74	2.40		+			+		
M. L.	9		17.4	21.3	3.70	2.97		+			+		
S. R.	13	♂	9.9	20.1	6.78	2.26			+		+		
A. Z.	15	♂	28.0	—	1.60	—		+			+		
Ch. M.	14	♂	11.0	19.8	2.48	2.69		+				+	
P. B.	11		19.2	11.7	2.32	4.33			+		+		
K. An.	8	♂	23.4	20.7	1.78	2.23	+				+		
C. E.	15	♂	10.8	18.6	4.75	3.29	+				+	+	
Arithmetic mean			20.1	18.5	2.98	2.90	Total	3	3	1		1	2



with nephropathy in the course of Schonlein Henoch disease

Poorest therapeutic results were obtained in the small group of children suffering from pyelonephritis

The influence of immunosuppressive therapy on some bone marrow elements is illustrated in Table 2

In group A percentages of erythroblasts in the bone marrow before treatment were low exceeding 12% in only one case. After treatment, a 2-4 fold increase in the percentage of erythroblasts present occurred in six cases

The G/E ratio of erythroblasts in group A show a different range of values before and after treatment increasing only in two cases. For the graphic presentation the method of ogives i.e. of cumulative frequencies curves was used (Fig 1)

The rise in the percentage of erythroblasts was due mainly to an increase in the relative numbers of basophilic and polychromatic erythroblasts while the percentage of eosinophilic erythroblasts remained unchanged

Changes in the granulocytic system under the influence of the immunosuppressive therapy (Table 2) consisted mainly in a slight drop of their percentages but all the values lie within normal limits. The ratio of granulocytes to

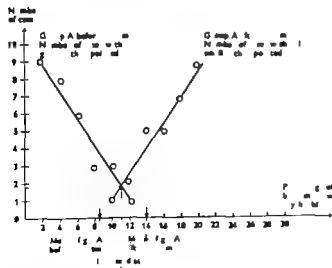


Fig 1 Cumulative curves of bone marrow erythroblasts in group A before and after immunosuppressive therapy

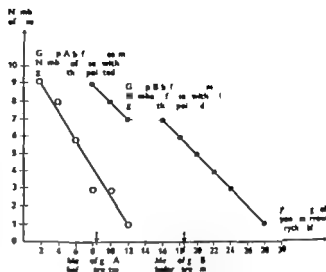


Fig 2 Cumulative curves of bone marrow erythroblasts in group A and B before immunosuppressive therapy

erythroblasts which had been elevated before treatment, tended to become normal after treatment in nearly all cases

As compared to group A the percentages of erythroblasts in group B were higher before treatment (Fig 2)

Under the influence of immunosuppressive therapy the percentages of erythroblasts in the bone marrow in group B behaved differently. A distinct rise occurred in two cases in which erythroblasts percentages were low before therapy (Sz R 11 years and C Z 11 years)

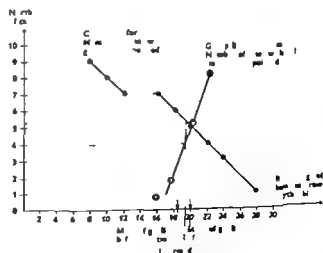


Fig 3 Cumulative curves of bone marrow erythroblasts in group B before and after immunosuppressive therapy

## DISCUSSION

Immunosuppressive therapy gave encouraging results in children who failed to improve after corticosteroid therapy and in whom prognosis was poor.

The therapeutic results observed in the primary nephrotic syndrome are in agreement with the findings of other authors (7, 12, 15). The more favorable results obtained for chronic glomerulonephritis were probably due to shorter duration of the disease process in our patients. Our observations indicate that immunosuppressant therapy should start with low doses of 6 MP (2.5 mg/kg/48 hr) which exert beneficial effects with almost no toxicity. We cannot agree with Shearn (16) and Grape (8) who administered high daily doses of 6 MP provoking toxic symptoms in all cases. Harmful effects of 6 MP on the peripheral blood and liver were observed in only 2 of our patients both of whom failed to improve after immunosuppressive therapy. This observation seems to correspond to the observations of Halikowski *et al.* (10) who noted that in acute leukemia children in whom drugs elicited toxic symptoms were usually refractory to treatment.

Hematologic studies in our patients confirmed the phenomenon (2, 3, 4, 6) of a lack of correlation between percentages of erythroblasts in the bone marrow and the degree of anemia. Similar degrees of anemia were observed in children with low numbers of erythroblasts in the bone marrow (group A) and in children with normal erythroblast percentages (group B).

In children with the nephrotic syndrome low percentages of erythroblasts in the bone marrow increased after immunosuppressive therapy but anemia has not improved.

On the basis of the observed facts the hypothesis may be proposed that at least two factors influence the erythropoietic system in the bone marrow. Presumably in the nephrotic syndrome there is a deficiency of erythropoietin I which stimulates multiplication of erythroblasts in the bone marrow while activity of erythropoietin II which regulates maturation

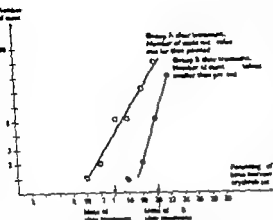


Fig 4 Cumulative curves of bone marrow erythroblasts in Group A and B after immunosuppressive therapy.

It can be seen in Fig 3 that the percentages of erythroblasts in group B before and after treatment were similar.

Despite of the increase in erythroblasts in the bone marrow after therapy in group A their values remained lower than in group B (Fig 4).

The granulocytes in patients of group B were affected little by the treatment. In all cases the bone marrow was moderately or richly cellular.

Hemoglobin levels and red blood cell counts were similar in both groups of children. Moderate normochromic anemia was observed.

In group A granulocytes counts which were elevated before treatment (mean 8200/mm<sup>3</sup>) dropped after treatment in all cases except one but neutropenia was not observed (mean 5700/mm<sup>3</sup>). Mean numbers of granulocytes in this group were higher than in group B even after treatment. In group B granulocyte counts were normal with a tendency to decrease after treatment.

Blood platelet counts in both groups were within normal limits with a slight tendency to decrease after treatment.

Reticulocyte counts performed in most cases ranged between 0.3-0.9%. In group A a slight increase was observed after treatment and in group B there was no change.

of erythroblasts, production of erythrocytes and synthesis of hemoglobin, is normal. This may be suspected on the basis of the low percentages of erythroblasts in the bone marrow and only moderate anemia. In glomerulonephritis the opposite was observed: anemia of the same degree was accompanied by normal numbers of erythroblasts in the bone marrow, suggesting deficiency of erythropoietin II but normal or enhanced activity of erythropoietin I.

At present the influence of immunosuppressive therapy on bone marrow erythroblasts is difficult to explain.

## SUMMARY

Immunosuppressive therapy (6 Mercaptopurine) has been used in 33 children with chronic renal diseases resistant to other therapeutic methods. Peripheral blood counts were done in all the children and in 18 of them bone marrow examinations were performed.

The results of immunosuppressive therapy were encouraging: many complete and partial remissions being obtained.

Children with nephrotic syndrome showed low percentages of erythroblasts in bone marrow. This was not observed in children suffering from chronic glomerulo- and pyelonephritis. Immunosuppressive therapy provoked increase of erythroblasts in bone marrow of children with nephrotic syndrome.

## REFERENCES

- Adams D A, Gordon A & Maxwell M H. Azathioprine treatment of immunological renal disease. *JAMA* 199: 459 1967.
- Alekseyev G I. The typical reactions of bone marrow in renal diseases. The mechanism of destroying bone marrow production. (In Russian) *Ter Arkh* 22: 58 1950.
- Bock H E, Nieth H & Solth K. Anämie bei Niereninsuffizienz. *Deutsch Med Wochr* 87: 573 1962.
- Callen I R & Limarin L R. Blood and bone marrow studies in renal disease. *Amer J Clin Path* 20: 3 1950.
- Chapal J, Jern R, Bonnet H, Navarro M & Lcoret R. Intérêt du chloramphénicol dans le traitement de certains syndromes néphrotiques. *Arch Franc Pédiat* 22: 473 1965.
- Choremis C, Megias H, Luzzonati A & Michiel S. Bone marrow studies in acute glomerulonephritis. *Helv Paediat Acta* 17: 138 1962.
- Farniaux J P. Le traitement du syndrome néphrotique idiopathique. Règles générales. *La Médecine Infantile* 74: 655 1967.
- Grupe W F & Heymann W. Cytotoxic drugs in steroid resistant renal disease. *Amer J Dis Child* 112: 448 1966.
- Halikowski B, Kucharska A & Sinczewicz-Pach A. Clinical results of immunosuppressive therapy in nephrotic syndrome and nephritis in children. (In Polish) *Pediat Pol* in press.
- Halikowski B, Armata J, Garwacz S, Wyżłowski J & Cykalis E. Drug sensitivity and resistance in acute leukemia in children and toxic action of the applied drugs. (In Polish) *Pediat Pol* 43: 669 1968.
- Japa J, Zinger Z, Dzeza I & Rozel A. Up to date results of treatment with cyclophosphamide (endoxan). (In Polish) *Pol Arch Med Intern* 38: 431 1967.
- Laguer G, Binety J, Halpern B & Millicz P. Les immuno-dépresseurs en néphrologie: essai de traitement des glomerulopathies chroniques primitives. *Presse Med* 75: 1779 1967.
- Laguer G, Binety J, Charlorbe P, Vassil J & Millicz P. La chimiothérapie dite immuno-dépresseur dans les syndromes néphrotiques primitifs de l'enfant. *Presse Med* 75: 1773 1967.
- Perlinski-Szczygler L & Gutka A. Nitrograulogen therapy in nephrotic syndrome. (In Polish) *Pol Tyg Lek* 20: 144 1965.
- Sivieri A M & Crawford J D. The treatment of nephrosis. *New Engl J Med* 272: 522 1965.
- Shearn M A. Mercaptopurine in the treatment of steroid resistant nephrotic syndrome. *New Engl J Med* 273: 943 1966.
- West C D, Hong R & Holliday N H. Effect of cyclophosphamide on lipid nephrosis in the human and aminonucleoside nephrosis in the rat. *J Pediat* 68: 516 1966.
- West C. Use of combined hormone and methchlorothamine (Nitrogen Mustard) therapy in lipid nephrosis. *Amer J Dis Child* 95: 495 1958.

Submitted June 7 1969

Accepted June 30 1969

Pediatric Clinic II  
Pediatric Institute  
Kraków-Prokocim  
Poland

Key words: Immunosuppressive therapy; chronic renal diseases in children; erythroblasts.

## HEMOGLOBIN HEMATOCRIT AND RED BLOOD CELL COUNT IN CAPILLARY (SKIN PRICK) BLOOD COMPARED TO VENOUS BLOOD IN CHILDREN

PETER JOHAN MOE

From the University of Bergen School of Medicine Department of Paediatrics Bergen Norway

Usually no distinction is made in the literature between figures for hemoglobin (Hb) hematocrit (Htc) and red blood cell count (RBC) in capillary and venous blood in children. Most textbooks for instance present data for normal Hb Htc and RBC without reference to the type of blood studied. Some of the data for normal blood values in infancy and childhood are actually from venous blood studies (2, 5, 6). In newborn infants skin prick samples from the heel have higher Hb than samples taken simultaneously from vein (8). It seems important to know whether there also in children may be a regular difference between venous Hb Htc and RBC and the corresponding figures in capillary (skin prick) blood obtained with a standardized technique used in clinical practice.

### MATERIAL AND METHODS

The material consists of 30 children, aged 2 to 13 years, admitted to the Children's Hospital. Blood studies were also performed on 30 adults (15 males and 15 females) members of the staff at the hospital.

Capillary blood was obtained from a 3 mm deep puncture in the finger with a "Medipoint" blood lancet. The first drop or two was discarded. Slight pressure had usually to be made some distance from the skin prick. None of the studied subjects had cyanotic or cold fingers and it was therefore not considered necessary to warm the finger in warm water before the puncture. Venous blood samples were obtained from an antecubital vein, the external jugular vein was used in one child. The application of a

tourniquet was found necessary in order to obtain the samples from the antecubital vein.

Hb Htc and RBC were determined in duplicate by methods reported previously (7).

### RESULTS

Higher Hb Htc and RBC were found in venous blood in children as well as in adults (Table 1). The difference seems to be most marked in children with a mean difference of Hb of 0.5 g/100 ml, a mean difference of Htc of 1.7 vol per cent and a mean difference of RBC of 0.18 mil/mm<sup>3</sup>. In adults there was only a difference in RBC of 0.08 (males) and 0.10 (females) mil/mm<sup>3</sup>.

In children higher venous Hb was found in 26 of the 30 cases, higher venous Htc in 21 of 28 cases and higher RBC in 25 of 29 studied cases.

The differences between Hb Htc and RBC in venous versus capillary blood were all significant at the 0.1 per cent level in children. The difference between Htc in venous versus capillary blood was significant at the 0.1 per cent level in adult females and between Hb at the 0.5 per cent level in both adult males and females while no significant difference was found between Htc in adult males or between RBC in both groups of adults.

### DISCUSSION

This study shows that a significant difference may exist in children between Hb Htc and

Table 1 Comparison between venous and capillary blood values

	Hemoglobin		Hematocrit		Red blood cells	
	No of cases	Mean (g/100 ml)	No of cases	Mean (vol %)	No of cases	Mean (mill/mm <sup>3</sup> )
Children						
Venous	30	12.63	28	39.1	29	4.51
Capillary	30	12.13	28	37.4	29	4.33
Difference		0.50		1.7		0.18
Adult males						
Venous	15	14.78	14	45.7	12	4.79
Capillary	15	14.43	14	44.7	12	4.71
Difference		0.35		1.0		0.08
Adult females						
Venous	15	13.19	15	41.8	14	4.33
Capillary	15	12.89	15	40.2	14	4.23
Difference		0.30		1.6		0.10

RBC in venous blood and blood from a finger prick. The observed difference in the means in the children about 4 per cent of the venous blood values are too high to be ignored. It seems also important to be aware of the possible error introduced by using the results of venous and capillary blood determinations interchangeably in a child. A difference between capillary and venous Hb of 1.3 g/100 ml, Htc of 3.5 vol per cent and RBC of 0.38 mill/mm<sup>3</sup> was found in the same child.

The method of using venous blood for determining Hb, Htc and RBC is according to Wintrobe (9) definitely more accurate than the use of capillary blood. Care must be taken however to avoid any error introduced by congestion of the arm. This may be difficult to avoid in a child. Furthermore it is out of the question to perform Hb determination on venous blood as a routine procedure even in children who are inpatients.

It is not quite clear whether Hb, Htc and RBC of venous and capillary blood are the same. (3) Studies by Gibson actually indicate that Hb, Htc and RBC of true capillary blood may be significantly less than those of venous blood.

Freely flowing blood obtained by skin puncture is probably more nearly arteriolar in composition than capillary. (3) Andresen & Mug

rage (1) found a close agreement between Hb, Htc and RBC in venous blood and blood from the finger pulp in children when a puncture wound deeply enough to give free blood flow was made and approximately 0.5 ml of blood collected in each case. Thus it is obvious that smaller difference may be obtained in children between venous and peripheral blood values than in this study if proper precautions are taken to secure a freely flowing sample in every case. This, however, seems to be impossible in pediatric practice. Anyhow this study has been performed with a technique widely used today.

### SUMMARY

Hb, Htc and RBC in capillary (skin prick) and venous blood have been compared in 30 children and in 30 adults (15 males and 15 females). Capillary blood was obtained from a 3 mm deep puncture in the finger with a Medipoint blood lancet. Higher mean values were found in venous blood particularly in the children.

### REFERENCES

1. Andresen M I & Mugrage E R. Venous and peripheral blood cell values. *Amer J Clin Path* 8: 46, 1938.
2. Baar H S, Baar S, Rogers K H & Stransky

R. R. *Disorders of blood forming organs in childhood* S. Karger Basel and New York 1963

Dacie J & Lewis S M. *Practical Haematology* J & A Churchill Ltd London 1963 3rd edition p 10

Gibson J G Seigman A. M Pascock W C Aub J C Fine J & Evans R D The distribution of red blood cells and plasma in large and minute vessels of the normal dog determined by radioactive isotopes of iron and iodine *J Clin Invest* 25 842 1946

Gunn G M Brown E W & Leiby M E Normal blood values in infancy and childhood *Pediatr Clin N Amer* 4 357 1957

Macle J B *Laboratory Medicine Hematology* The C V Mosby Company ST Louis 1967 3rd edition

Moe P J Normal red blood picture during the

first three years of life *Acta Paediatr Scand* 54 69 1965

- 8 Mollison P L. *Blood transfusion in clinical practice* The C V Mosby Company St Louis 1967 3rd edition p 379
- 9 Wintrobe M M *Clinical hematology* H Lippincott London 1967 6th edition p 411

Submitted April 14 1969

Accepted June 9 1969

Dept of Paediatrics  
Haukeland sykehus  
Bergen  
Norway

*Key words* Capillary\* (skin prick) blood venous blood hemoglobin hematocrit red blood cell count

Table 1 Comparison between venous and "capillary" blood values

	Hemoglobin		Hematocrit		Red blood cells	
	No of cases	Mean (g/100 ml)	No of cases	Mean (vol %)	No of cases	Mean (mill/mm <sup>3</sup> )
Children						
Venous	30	12.63	28	39.1	29	4.51
Capillary	30	12.13	28	37.4	29	4.33
Difference		0.50		1.7		0.18
Adult males						
Venous	15	14.78	14	45.7	12	4.79
Capillary	15	14.43	14	44.7	12	4.71
Difference		0.35		1.0		0.08
Adult females						
Venous	15	13.19	15	41.8	14	4.33
Capillary	15	12.89	15	40.2	14	4.23
Difference		0.30		1.6		0.10

RBC in venous blood and blood from a finger prick. The observed difference in the means in the children about 4 per cent of the venous blood values are too high to be ignored. It seems also important to be aware of the possible error introduced by using the results of venous and capillary blood determinations interchangeably in a child. A difference between capillary and venous Hb of 1.3 g/100 ml, Htc of 3.5 vol per cent and RBC of 0.38 mill/mm<sup>3</sup> was found in the same child.

The method of using venous blood for determining Hb, Htc and RBC is, according to Wintrobe (9), definitely more accurate than the use of capillary blood. Care must be taken however to avoid any error introduced by congestion of the arm. This may be difficult to avoid in a child. Furthermore, it is out of the question to perform Hb determination on venous blood as a routine procedure even in children who are inpatients.

It is not quite clear whether Hb, Htc and RBC of venous and capillary blood are the same (3). Studies by Gibson actually indicate that Hb, Htc and RBC of true capillary blood may be significantly less than those of venous blood.

Freely flowing blood obtained by skin puncture is probably more nearly arteriolar in composition than capillary (3). Andresen & Mug-

rage (1) found a close agreement between Hb, Htc and RBC in venous blood and blood from the finger pulp in children when a puncture wound deeply enough to give free blood flow was made and approximately 0.5 ml of blood collected in each case. Thus it is obvious that smaller difference may be obtained in children between venous and peripheral blood values than in this study if proper precautions are taken to secure a freely flowing sample in every case. This however seems to be impossible in pediatric practice. Anyhow, this study has been performed with a technique widely used today.

### SUMMARY

Hb, Htc and RBC in capillary (skin prick) and venous blood have been compared in 30 children and in 30 adults (15 males and 15 females). Capillary blood was obtained from a 3 mm deep puncture in the finger with a Medipoint blood lancet. Higher mean values were found in venous blood particularly in the children.

### REFERENCES

1. Andresen M. I. & Mugrage E. R. Venous and peripheral blood cell values. *Amer J Clin Path* 8: 46, 1938.
2. Baar H. S., Baar S., Rogers K. B. & Stramsky

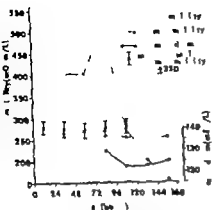


Fig 1 Serum and urine osmolalities and serum sodium in case 1. Note the hypertonic urine and low serum sodium and osmolality.

were no seizures hydration appeared normal and there was no edema.

Because of another study then in progress in which serum and urine osmolality were being measured in newborns serum and urine were collected daily, frozen and stored. Several weeks later osmolality was measured. The results of these sequential determinations are shown in Fig 1. Although the initial serum values of 264 and 270 mOsm/l on days 2 and 4 were within normal limits thereafter below normal values (7) of 254 mOsm/l were obtained on each of days 5, 6 and 7. The serum sodium values of 135 mEq/l on day 4 and 120-125 mEq/l on days 5-7 reflect this change.

The patient's urine was more concentrated than that of control babies fed the same formula rising to 513 mOsm/l on the fourth day. The serum urine osmolality of 21 normally hydrated 4 day old new-

born was 118 mOsm/l with a range of 49-363 mOsm/l (7). A blood urea nitrogen (BUN) of 16.5 on the fourth day indicated that dehydration or diminished glomerular filtration rate did not play a role in producing the concentrated urine. McCance & Widdowson have shown that the mean BUN of normal 4 day-old infants fed breast milk was 29.1 mg/100 ml (15).

After the initial asphyxial episode the baby appeared well, he was discharged on the seventh day apparently in good health. At that time the urine had become dilute (122 mOsm/l). No specific therapy had been instituted since the results of the osmolality studies were known only after the baby had been discharged.

### Case 2

This 2285 g male infant was the product of a 42 week gestation. Because of prolonged labor delivery was by Caesarian section. The infant was pale, blue and flaccid at birth. No resuscitation was performed. On the third day dehydration and a temperature of 100.3 F were noted and he was transferred to the Montreal Children's Hospital. On admission the baby appeared dehydrated and fatally malnourished. The weight was 2040 g. The serum sodium was 123 mEq/l, the potassium 6 mEq/l, the calcium 7 mg/100 ml, the glucose 144 mg/100 ml, the BUN 52 mg/100 ml. There was a mild compensated metabolic acidosis. The urine osmolality was 470 mOsm/l. Investigations due to E. coli was diagnosed and anti-microbial therapy was started. A single brief seizure was noted on the day of admission and a bulging fontanel was present for one week. Values for serum sodium, urine osmolality and sodium intake are shown in Fig 2a. For the first 24 hours after admission the serum sodium remained between 120-125 mEq/l despite intravenous administration of a solution of sodium chloride (50 mEq/l) calculated to give 10-12 mEq/day. On the fourth day of life 12 mEq of hypertonic sodium chloride solution (400 mEq/l) were given over a 2-hour period. This was calculated to elevate the serum sodium to 133 mEq/l and was in addition to the 12 mEq infused over the total 24-hour period. The serum sodium rose transiently to 135 mEq/l. 16 hours later it had fallen to 125 mEq/l.

The combination of persistent hyponatremia by peritoneal urine and normal renal function suggested the possibility of an inappropriate ADH syndrome. Fluids were withheld for an 8-hour period on the fifth day of life and the serum sodium rose to 135 mEq/l. A water loading test, as described was done on the sixth day. At that time the BUN was 26 mg/100 ml. On the seventh day the baby developed diarrhea and 10-12 mEq of sodium per day which previously had no effect on the hyponatremia was then sufficient to maintain a normal serum sodium. The urine became dilute on the eighth day of life. The weight varied between 2220 and 2350 g from the fifth to the thirteenth day when a gradual and sustained weight gain was seen.

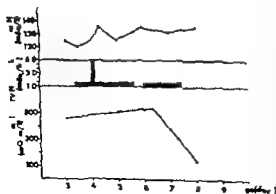


Fig 2a Serum sodium, intravenous sodium intake and urine osmolality in case 2. Note the hypertonic urine and transient rise in serum sodium on day 4 after hypertonic sodium infusion.



## HYPONATREMIA FOLLOWING ASPHYXIA NEONATORUM

WILLIAM FELDMAN KEITH N. DRUMMOND and MICHAEL KLEIN\*

*From the Renal Laboratory McGill University-Montreal Children's Hospital Research Institute and the Neonatal Unit Department of Obstetrics and Gynecology Royal Victoria Hospital Montreal Canada*

Hyponatremia in the newborn may result from adrenal insufficiency salt losing virilizing adrenal hyperplasia water intoxication or renal disease. Maternal hyponatremia during labor can also lead to hyponatremia in newborns (21).

Schwartz and his associates first recognized the syndrome of inappropriate secretion of antidiuretic hormone (ADH) in hyponatremic adults with bronchogenic carcinoma (20). Briefly this syndrome is characterized by hyponatremia with hypertonic urine in the absence of renal, adrenal, cardiac or hepatic disease. The hyponatremia is not corrected by the administration of hypertonic saline but is corrected by fluid restriction.

Since Schwartz's description this syndrome

has been reported in patients with intracranial disease (6), myxedema (11), acute intermittent porphyria (13), pulmonary tuberculosis (24) and Vincristine therapy (8). McCrory & MacAulay reported a brain damaged 5 month old infant whose hyponatremia was associated with high blood levels of ADH (18). To our knowledge their patient is the youngest described with this syndrome.

The purpose of this report is to describe 3 newborn with asphyxia neonatorum whose hyponatremia appears best explained by an inappropriate secretion of ADH.

### METHODS

Blood was taken by femoral venepuncture in cases 1 and 2 and from a warmed heel in case 3. Urine was collected in disposable plastic containers and samples were analyzed within 2 hours or frozen for later determination. In case 3 urine was collected on a metabolic bed. Osmolality was measured with a Fiske osmometer\* using 0.2 ml samples. Reproducibility with this instrument in our laboratory is  $\pm 0.5$  mOsm/l.

### CASE REPORTS

#### Case 1

This male infant weighing 4182 g at birth was the product of a 40 week gestation. Delivery was by Cesarean section because of cephalopelvic disproportion. The condition at birth was poor; he was cyanosed, flaccid and required intubation. The Apgar score at one minute was 2. Shortly after intubation and manual positive pressure oxygen therapy the color and tone improved. Thereafter the infant appeared well and took oral feedings adequately. There

\*Medical Research Council of Canada Fellow 1966-1967. Present address: Department of Pediatrics, McMaster University, Hamilton, Ontario.

\*Associate Professor of Pediatrics, McGill University and Physician and Director, Renal Laboratory, Montreal Children's Hospital.

Senior Resident in Neonatology, Royal Victoria Hospital. Present address: Chief Resident in Pediatric Medicine, Montreal Children's Hospital.

Sterilon Corp., Buffalo, N.Y.

Fiske Associates Inc., Uxbridge, Massachusetts.

In our nursery babies are offered 30-45 ml of glucose 5% in water every 4 hours for the first 3 feedings; thereafter a commercial low solute cow's milk preparation (Enfalac 20 calories/30 ml—Mead Johnson Canada Ltd., Toronto, Ontario) is given at 4 hourly intervals beginning with about 60 ml/feeding on the second day and increasing to about 100-120 ml by the seventh day.

## DISCUSSION

The salient features of the syndrome of inappropriate secretion of ADH have been summarized by Haden & Knox (12). They include

- 1 Hyponatremia of moderate to marked degree without significant renal, adrenal, hepatic or cardiac disease
- 2 Excretion of a hypertonic urine
- 3 Normal or low blood urea nitrogen
- 4 Urinary loss of administered sodium
- 5 Inability to maintain normal serum sodium concentrations by infusion of hypertonic saline
- 6 Correction of hyponatremia by fluid restriction

ADH production is termed inappropriate in this syndrome because the secretion of ADH is normally inhibited by hypotonic plasma. This inhibition causes the free water clearance to rise leading to the excretion of a dilute urine thus permitting plasma osmolality to rise to normal levels. The excretion of hypertonic urine in the face of hypotonic plasma and normal renal function implies that there is continuing ADH secretion which is inappropriate for the level of serum osmolality.

Cases 1 and 2 manifested most of the features of this syndrome. A hypertonic urine was elaborated in the presence of hyponatremia and in the absence of demonstrable renal, adrenal, hepatic or cardiac disease. In case 2 the administration of hypertonic saline produced only a transient elevation of serum sodium. Although urine sodium was not measured it is possible that the rapid decline in serum sodium following hypertonic saline infusion was due to urinary loss. The urinary loss of administered sodium is considered to be related to a decrease in aldosterone secretion secondary to an expanded extracellular fluid volume (20). Case 3 presents some problems with respect to the diagnosis as defined by Haden & Knox (12). The syndrome as described in adults requires the absence of renal and cardiac dis-

Blood gases from a warmed heel at one hour of age showed a severe combined respiratory and metabolic acidosis pH 7.135 PCO<sub>2</sub> 50 mm Hg base excess -12.1 mEq/l and PO<sub>2</sub> in room air was 35 mm Hg. Blood sugar prior to oral or intravenous feedings was 180 mg/100 ml. Intramuscular chloramphenicol was begun at 25 mg/kg/day. Chest X-ray showed moderate cardiomegaly with severe vascular congestion. Electrocardiogram showed a right axis deviation. At 3 hours of age an intravenous drip of 10% glucose and water was begun at 65 ml/kg/day containing 15 mEq/day of sodium bicarbonate. The liver was 5 cm below the costal margin. At 7 hours of age the child was put in 50% oxygen and was desaturated because of persistent cardiomegaly and pulmonary vascular congestion. He was given an additional 7.5 mEq of sodium bicarbonate intravenously over 2 hours and was started on hydrocortisone (7.5 mg every 4 hours for 6 doses). At 28 hours the infant had a generalized seizure. The fontanel was not bulging. The blood sugar was 229 mg/100 ml. At 24 hours of age the liver was 2 cm below the costal margin and the child was no longer in heart failure and his colour and tone were much improved.

At 37 hours the patient was lethargic and edematous. The weight had increased from 2940 g at birth to 3050 g. The serum sodium had fallen from 135 mEq/l at 12 hours of age to 128 mEq/l and urine output was negligible. A diagnosis of inappropriate ADH response was entertained. A repeat serum sodium at 41 hours was 124 mEq/l with a serum osmolality of 252 mOsm/l. Simultaneous urine osmolality was 559 mOsm/l. The urine sodium on this sample was 4.5 mEq/l and urine sodium determinations during the following 2 days ranged from 0.5 mEq/l to 13.5 mEq/l. The latter following an infusion of hypertonic saline. Non protein nitrogen was 75 mg/100 ml.

Since the child had continuing central nervous system signs it was elected to stop intravenous fluids and attempt to reduce a diuresis with an osmotic agent. Oral glycerol was given (1 g/kg) by nasogastric tube (?). Four hours later the patient had voided only 10 ml of hyposmolar (325 mOsm/l) urine. The serum sodium had dropped further to 170 mEq/l. The serum osmolality however had risen from 252 to 277 mOsm/l. The patient appeared more alert. By 7 hours following the glycerol the patient had voided 23 ml. The serum sodium was 132 mEq/l and the serum osmolality 289 mOsm/l. Sixteen hours after glycerol administration the serum sodium had risen to 138 mEq/l with an accompanying serum osmolality of 266 mOsm/l. The total diuresis was 194 ml. The central nervous system status was markedly improved and the child had lost 40 gm during the 30 hour period following glycerol administration despite an intake of 120 ml of milk. The subsequent course was unremarkable. The lowest urine osmolality (110 mOsm/l) was recorded on the seventh day. Non protein nitrogen at that time was 25. Follow up examination at 4 months revealed a healthy infant.

## Water Load Studies (Fig 2 b)

Weil noted that infants given a water load of 30–50 ml/kg produced a peak diuresis in 50–120 min (23). McCrory found that a normal 5 month old infant diluted his urine one hour after a water load, whereas a patient with high ADH levels did not dilute his urine until 3 hours after the load (18). In the absence of renal, hepatic, adrenal or cardiac dysfunction the ability to deliver a water load depends on the ability to suppress ADH secretion. If a water load cannot be delivered in a normal manner one can assume an inability to suppress ADH.

The following water load study was carried out in case 2 on the sixth and fifteenth days, 2 normal 6 day old infants served as controls. Baseline serum and urine were taken for osmolality just prior to a scheduled feeding. Thirty millilitres per kilogram of 5% glucose in water was then given by gavage over a 5–10 min period instead of the feeding. Osmolality was determined on each urine voided over the next 6 hours and on serum specimens taken at hourly intervals. In the controls a fall in serum osmolality of 4 mOsm/l was seen at one hour with a return to pre test levels by 2 hours. In the first study in case 2 a slightly greater fall (8 mOsm/l) was seen at one hour and pre test levels had not been reached by 4 hours.

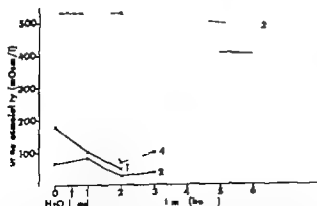


Fig 2 b Changes in urine osmolality after water loading in normal 6 day old infants and in case 2 at age 6 and 15 days. Note the decrease in urine osmolality at 2 hours in the controls and in the patient at 15 days. Note also the persistently hyperosmolar urine for up to 6 hours in case 2 at age 6 days.

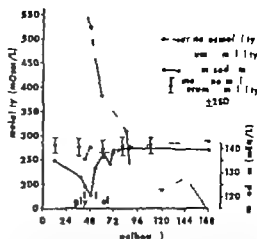


Fig 3 a Case 3 Serum sodium and serum and urine osmolality. Note the high urine and low serum osmolality and the fall in serum sodium paralleling the usual increase in serum osmolality.

By 2 hours urine osmolality had decreased 50% in the controls. Although a similar response was seen in case 2 at day 15 the urine osmolality remained greater than 500 mOsm/l as late as 6 hours following the water load performed on the sixth day.

## Case 3 (Fig 3)

This full term 2940 g male infant was born after a 16 hour labor to a gravida 1 mother. Membranes were ruptured artificially 6 hours prior to delivery and meconium staining of the amniotic fluid was noted. There were no other signs of fetal distress. At birth the infant was asphyxiated and meconium stained. Positive pressure ventilation was given with bag and mask until spontaneous respirations were sustained at 2 / min. The Apgar score at one minute was 3 and at 5 minutes 6, at which time the heart rate was 100. The baby was still cyanotic, pale, flaccid with a weak cry, grunting respirations and marked subcostal retractions.

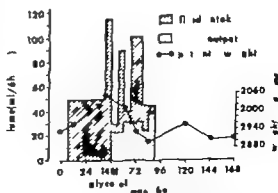


Fig 3 b Case 3 Fluid intake and output and weight changes. Note the diuresis and weight loss after glycerol administration despite continued oral intake.

## DISCUSSION

The salient features of the syndrome of inappropriate secretion of ADH have been summarized by Haden & Knox (12). They include

- 1 Hyponatremia of moderate to marked degree without significant renal, adrenal, hepatic or cardiac disease
- 2 Excretion of a hypertonic urine
- 3 Normal or low blood urea nitrogen
- 4 Urinary loss of administered sodium
- 5 Inability to maintain normal serum sodium concentrations by infusion of hypertonic saline
- 6 Correction of hyponatremia by fluid restriction

ADH production is termed inappropriate in this syndrome because the secretion of ADH is normally inhibited by hypotonic plasma. This inhibition causes the free water clearance to rise leading to the excretion of a dilute urine thus permitting plasma osmolality to rise to normal levels. The excretion of hypertonic urine in the face of hypotonic plasma and normal renal function implies that there is continuing ADH secretion which is inappropriate for the level of serum osmolality.

Cases 1 and 2 manifested most of the features of this syndrome. A hypertonic urine was elaborated in the presence of hyponatremia and in the absence of demonstrable renal and renal hepatic or cardiac disease. In case 2 the administration of hypertonic saline produced only a transient elevation of serum sodium. Although urine sodium was not measured it is possible that the rapid decline in serum sodium following hypertonic saline infusion was due to urinary loss. The urinary loss of administered sodium is considered to be related to a decrease in aldosterone secretion secondary to an expanded extracellular fluid volume (20). Case 3 presents some problems with respect to the diagnosis as defined by Haden & Knox (12). The syndrome as described in adults requires the absence of renal and cardiac dis-

Blood gases from a warmed heel at one hour of age showed a severe combined respiratory and metabolic acidosis pH 7.135 PCO<sub>2</sub> 59 mm Hg base excess -12.1 mEq/l and PO<sub>2</sub> in room air was 35 mm Hg. Blood sugar prior to oral or intravenous feedings was 180 mg/100 ml. Intramuscular chloromycetin was begun at 25 mg/kg/day. Chest X-ray showed moderate cardiomegaly with severe vascular congestion. Electrocardiogram showed a right axis deviation. At 3 hours of age an intravenous drip of 10% glucose and water was begun at 65 ml/kg/day containing 15 mEq/day of sodium bicarbonate. The liver was 5 cm below the costal margin. At 7 hours of age the child was put in 50% oxygen and was digitized because of persistent cardiomegaly and pulmonary vascular congestion. He was given an additional 7.5 mEq of sodium bicarbonate intravenously over 2 hours and was started on hydrocortisone (7.5 mg every 4 hours for 6 doses). At 28 hours the infant had a generalized seizure. The fontanel was not bulging. The blood sugar was 229 mg/100 ml. At 24 hours of age the liver was 2 cm below the costal margins and the child was no longer in heart failure and his colour and tone were much improved.

At 37 hours the patient was lethargic and edematous. The weight had increased from 2940 g at birth to 3050 g; the serum sodium had fallen from 135 mEq/l at 12 hours of age to 128 mEq/l and urine output was negligible. A diagnosis of inappropriate ADH response was entertained; a repeat serum sodium at 41 hours was 124 mEq/l with a serum osmolality of 252 mOsm/l. Simultaneous urine osmolality was 559 mOsm/l. The urine sodium on this sample was 4.5 mEq/l and urine sodium determinations during the following 2 days ranged from 0.5 mEq/l to 13.25 mEq/l; the latter following an infusion of hypertonic saline. Non protein nitrogen was 75 mg/100 ml.

Since the child had continuing central nervous system signs it was decided to stop intravenous fluids and attempt to achieve a diuresis with an osmotic agent. Oral glycerol was given (1 g/kg) by nasogastric tube (?). Four hours later the patient had voided only 10 ml of hyperosmolar (523 mOsm/l) urine. The serum sodium had dropped further to 120 mEq/l. The serum osmolality however had risen from 252 to 271 mOsm/l. The patient appeared more alert. By 7 hours following the glycerol the patient had voided 23 ml the serum sodium was 13 mEq/l and the serum osmolality 69 mOsm/l. Within hours after glycerol administration the serum sodium had risen to 138 mEq/l with an accompanying serum osmolality of 66 mOsm/l. The total diuresis was 194 ml. The central nervous system signs were markedly improved and the child had lost 40 gm during the 30 hour period following glycerol administration despite an intake of 120 ml of milk. The subsequent course was unremarkable. The lowest urine osmolality (110 mOsm/l) was recorded on the seventh day. Non protein nitrogen at that time was 25. Follow up examination at 4 months revealed a healthy infant.

### Water Load Studies (Fig 2 b)

Weil noted that infants given a water load of 30–50 ml/kg produced a peak diuresis in 50–120 min (23). McCrory found that a normal 5 month old infant diluted his urine one hour after a water load, whereas a patient with high ADH levels did not dilute his urine until 3 hours after the load (18). In the absence of renal, hepatic, adrenal or cardiac dysfunction the ability to deliver a water load depends on the ability to suppress ADH secretion. If a water load cannot be delivered in a normal manner one can assume an inability to suppress ADH.

The following water load study was carried out in case 2 on the sixth and fifteenth days. 2 normal 6-day-old infants served as controls. Baseline serum and urine were taken for osmolality just prior to a scheduled feeding. Thirty millilitres per kilogram of 5% glucose in water was then given by gavage over a 5–10 min period instead of the feeding. Osmolality was determined on each urine voided over the next 6 hours and on serum specimens taken at hourly intervals. In the controls a fall in serum osmolality of 4 mOsm/l was seen at one hour with a return to pre test levels by 2 hours. In the first study in case 2 a slightly greater fall (8 mOsm/l) was seen at one hour and pre test levels had not been reached by 4 hours.

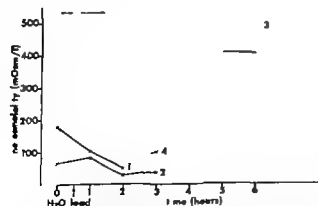


Fig 2 b Changes in urine osmolality after water loading in normal 6 day old infants and in case 2 at age 6 and 15 days. Note the decrease in urine osmolality at 2 hours in the controls and in the patient at 15 days. Note also the persistently hyperosmolar urine for up to 6 hours in case 2 at age 6 days.

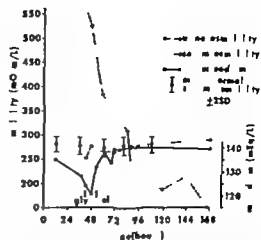


Fig 3 a Case 3 Serum sodium and serum and urine osmolality. Note the high urine and low serum osmolality and the fall in serum sodium paralleling the initial increase in serum osmolality.

By 2 hours urine osmolality had decreased 50% in the controls. Although a similar response was seen in case 2 at day 15 the urine osmolality remained greater than 500 mOsm/l as late as 6 hours following the water load performed on the sixth day.

### Case 3 (Fig 3)

This full term 2940 g male infant was born after a 16 hour labor to a gravid 1 mother. Membranes were ruptured artificially 6 hours prior to delivery and meconium staining of the amniotic fluid was noted. There were no other signs of fetal distress. At birth the infant was asphyxiated and meconium stained. Positive pressure ventilation was given with bag and mask until spontaneous respirations were sustained at 2 / min. The Apgar score at one minute was 3 and at 5 minutes 6, at which time the heart rate was 100. The baby was still cyanotic, pale, flaccid with a weak cry, grunting respirations and marked subcostal retractions.

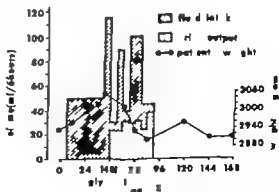


Fig 3 b Case 3 Fluid intake and output and weight changes. Note the diuresis and weight loss after glycerol administration despite continued oral intake.

- 1 Caspary, L., Arbus, L. & Lanerthes, Y. Note sur le traitement de l'œdème cérébral par le glycérol *Neurochirurgie* 11 343 1965
- 2 Castore, G., Gaudette, B. & Virao, M. Oral glycerol for the reduction of intracranial pressure *J Neurosurg* 21 278 1964
- 3 Carter, N. W., Rector, F. C. & Sedlin, D. W. Hyponatremia in cerebral disease resulting from inappropriate secretion of antidiuretic hormone *New Eng J Med* 264 67 1961
- 4 Feldman, W. & Drummond, A. D. Serum and urine osmolality in normal newborns (In press *Canad Med Ass J*)
- 5 Fine, R. N., Clarke, R. R. & Short, N. A. Hyponatremia and Vincristine therapy syndrome possibly resulting from inappropriate antidiuretic hormone secretion *Amer J Dis Child* 112 256 1966
- 6 Finkel, D. A., Pyle, H. R., Porter, J. C., Beard, A. G. & Pears, T. C. Control of water balance in the newborn *Amer J Dis Child* 106 157 1963
- 7 Goldberg, M. Hyponatremia and the inappropriate secretion of antidiuretic hormone (Editorial) *Amer J Med* 35 293 1963
- 8 Goldberg, M. & Rernch, M. Studies on the mechanism of hyponatremia and impaired water excretion in myxedema *Ann Intern Med* 56 120 1962
- 9 Haden, H. T. & Kaor, G. W. Cerebral hyponatremia with inappropriate antidiuretic hormone syndrome *Amer J Med Sci* 249 581 1965
- 10 Hoffman, H. S., Teichgraber, D. P. & Bartter, F. C. Abnormal electrolyte and water metabolism in acute intermittent porphyria *Amer J Med* 32 734 1966
- 11 Janovsky, M., Wertnick, J. & Stamatova, V. Antidiuretic activity in the plasma of human in tests after a load of sodium chloride *Acta Paediatr Scand* 54 543 1965
- 12 McCann, R. A. & Widdowson, I. M. Blood urea in the first days of life *Lancet* 11 797 1947
- 13 — Metabolism and renal function in the first two days of life *Sympos Quæst Biol* 19 161 1954
- 14 — Protein catabolism and renal function in the first two days of life in premature infants and multiple births *Arch Dis Child* 30 405 1955
- 15 McCrory, W. W. & Macculay, H. Idiopathic hyponatremia in an infant with diffuse cerebral damage *Pediatrics* 20 23 1957
- 16 Ober, Polleri, J. Ensayo de la glicerasa en el dano cerebral del recién nacido *Arch Pediatr Urug* 36 601 1965
- 17 Schwartz, W. B., Bennett, W., Corelop, S. & Bartter, F. C. Syndrome of renal sodium loss and hyponatremia probably resulting from inappropriate secretion of antidiuretic hormone *Amer J Med* 23 529 1957
- 18 Strauss, J. Fluid and electrolyte composition of the fetus and the newborn *Pediatr Clin N Amer* 13 1077 1966
- 19 Thomson, J. Observations on the urine of the newborn infant *Arch Dis Child* 19 169 1944
- 20 Weil, W. E. The evaluation of renal function in infancy and childhood *Amer J Med Sci* 229 678 1955
- 21 Weiss, H. & Katz, S. Hyponatremia resulting from apparently inappropriate secretion of antidiuretic hormone in patients with pulmonary tuberculosis *Amer Rev Resp Dis* 92 609 1965

Submitted March 4 1968

Accepted May 17 1969

(L. N. D.) Montreal Children's Hospital  
Research Institute  
2300 Upper Street  
Montreal  
Canada

**Key words:** Hyponatremia antidiuretic hormone asphyxia neonatorum

case Case 3 had congestive failure secondary to severe asphyxia, although at the time hyponatremia and urine hypertonicity developed the cardiac problem was controlled. The presence of cardiomegaly and pulmonary vascular congestion is a major feature of severe asphyxia neonatorum (3); it may thus be impossible to dissociate the role of cardiac failure from that of inappropriate ADH secretion in such infants. The slight elevation of non protein nitrogen was probably due to pre renal causes (17).

The use of oral glycerol as an osmotic agent has been previously described (2, 4, 5, 19). In our patient glycerol administration was followed by a substantial diuresis which began 4 hours after the first dose and lasted 24 hours with weight loss despite continued oral intake. The initial drop in serum sodium from 124 to 120 mEq/l following glycerol administration in the face of a rising serum osmolality may represent an initial shift of fluid from intracellular to extracellular spaces prior to the onset of diuresis. The low urine sodium concentrations remain unexplained.

Using a rat bioassay Janovsky and others were unable to detect antidiuretic activity in the plasma of infants under 2 1/2 months (14). Ames however was able to detect ADH in the urine of newborns under 3 days of age after fluid deprivation (1). Because bioassay techniques are difficult to perform with reproducibility other workers have used indirect techniques to detect qualitative and quantitative ADH responses. These include production of a concentrated urine following hypertonic saline administration or fluid deprivation. Fisher using infusion of 5% sodium chloride showed that newborns could produce a concentrated urine (9). Thomson found that during the first 3 days of life infants were able to respond to a limited fluid intake by excreting a concentrated urine (22). The data of these workers may be interpreted as evidence in favour of ADH secretion in newborn infants.

The mechanism which leads to the inappropriate secretion of ADH is unknown. Most cases reported to date have had lesions of the

thorax or central nervous system. An irritative lesion involving thoracic volume receptors or central nervous system osmoreceptors has been postulated as a persisting stimulus for ADH secretion (10). The patients in the present report had asphyxia at birth. *E. coli* meningitis was also present in the second newborn. It is considered possible that an insult to the central nervous system in each infant served as a stimulus to ADH secretion. The observations of McCance & Widdowson that mature infants born after prolonged delivery or with asphyxia neonatorum may have smaller than normal urine volumes (16) is of interest in this context. It is suggested that this condition may be more common than suspected and that serum sodium levels and urine osmolality or specific gravity be assessed in newborns with asphyxia neonatorum or neonatal meningitis. Specific therapy for hyponatremia in this condition consists of fluid restriction rather than administration of hypertonic saline. Glycerol may be of value in those patients in whom central nervous system signs due to cerebral edema are evident.

## SUMMARY

Three newborns with transient asphyxia developed hyponatremia and hypertonic urine. Inappropriate secretion of ADH is proposed as a reasonable explanation for these findings. Serum sodium and urine concentration should be assessed in newborns with asphyxia or meningitis since this condition may be more common than realized.

## REFERENCES

1. Ames R. G. Urinary water excretion and neurohypophyseal function in full term and premature infants shortly after birth. *Pediatrics* 12: 272, 1953.
2. Buckell M. & Wahh L. Effect of glycerol by mouth on raised intracranial pressure in man. *Lancet* II 1151, 1964.
3. Burnard E. D. & James L. S. Future of the heart after undue asphyxia at birth. *Pediatrics* 28: 545, 1961.

Table 1 *Clinical and haematological data*

Case no	Age (months) and sex	Weight (kg) Degree of malnutrition	Serum proteins (g/100 ml) alb/glob	Haemoglobin (g/100 ml)	Serum iron Serum iron binding Cap. (μ/100 ml)
1	30/♂	6.3/IV	$7.8 = \frac{5.3}{2.5}$	6.75	—
2	36/♂	6.8/IV	$6.3 = \frac{3.8}{2.5}$	6.65	40/333
3	30/♀	6.71/IV	$6.5 = \frac{4.4}{2.1}$	8.45	—
4	18/♀	5.5/IV	$7.2 = \frac{4.6}{2.6}$	3.0	136/312.5
5	24/♂	5.83/IV	—	6.95	—
6	18/♀	6.38/III	$6.4 = \frac{4.2}{2.2}$	8.45	80/390
7	18/♂	6.7/III	$4.6 = \frac{1.5}{3.1}$	7.40	—
	27	10.5/II	$6.0 = \frac{3.8}{2.2}$	10.80	—
8	18/♂	6.24/III	$4.2 = \frac{2.2}{2.0}$	7.17	90/390
	23	10.8/—	$6.8 = \frac{4.2}{2.6}$	11.55	—
9	36/♀	8.5/III	$6.4 = \frac{4.4}{2.0}$	3.85	28/314
9	42	10/—	$5.6 = \frac{3.0}{2.6}$	12.30	41/417
10	9/♂	4.8/III	$3.4 = \frac{1.8}{1.6}$	9.20	—
11	10/♂	5.9/III	$6.1 = \frac{3.9}{2.2}$	10.05	—
12	14/♂	5.8/III	—	9.00	—
13	14/♂	7.5/II	$7 = \frac{5.5}{1.5}$	9.20	67/334
	19.5	10.1/II	—	10.80	—
14	30/♀	10.3/II	$4.5 = \frac{2.5}{2.0}$	6.95	64/350
15	12/♂	6.3/II	$7.5 = \frac{5.5}{2.0}$	9.50	—
16	30/♀	8.7/II	$6.5 = \frac{4.5}{2.0}$	11.55	—
17	24/	7.5/II	$6.8 = \frac{4.1}{2.7}$	11.40	—
18	18/♂	8.1/II	$4.8 = \frac{3.8}{1.0}$	12.30	82/390
19	12/	6.5/II	$6.6 = \frac{4.2}{2.4}$	9.6	93/346
20	18/♂	7.8/II	$6.8 = \frac{3.8}{3.0}$	11.25	—
21	36/	9.9/II	$4.8 = \frac{2.8}{2.0}$	5.45	42/408
	40	11.5/II	$6.2 = \frac{4.0}{2.2}$	10.05	—
22	70	8.7/II	$6.2 = \frac{4.0}{2.2}$	8.30	183/248
23	24/♂	9.3/I	$4 = \frac{2.2}{2.0}$	6.95	157/350
	27	II —	$7.0 = \frac{4.5}{2.5}$	11.85	—
24	36/♂	12/I	$6.2 = \frac{3.7}{2.5}$	9.65	49/350



## JEJUNAL MUCOSA IN INFANTILE MALNUTRITION

IZZET BERKEL ÖZDEN KIRAN and BURHAN SAY

*From Hacettepe University Faculty of Medicine Department of Pediatrics and Institute of Child Health Hacettepe Children's Hospital Ankara Turkey*

Malnutrition is still one of the major problems in developing countries (24). This is also a common condition in Turkey: a study carried out at this hospital in 1958 disclosed that 43 per cent of the children under two years of age seen at the out patient clinics had some degree of malnutrition (14).

Although the different aspects of infantile malnutrition have been extensively studied in recent years, histological studies of the small intestine in these patients were limited to autopsy material until the advent of per oral biopsy (12, 26-33). Recently using the per oral technique Stanfield *et al* and Burman Cook & Lee reported jejunal mucosal changes and disaccharidase activities in African children with kwashiorkor (6, 10, 31). Brunser *et al* described jejunal mucosa of malnourished infants with special reference to the mitotic index (5). Since we could find no reports on the histologic characteristics and disaccharidase levels of the intestinal mucosa in infants with marasmus, we are presenting here the results of our study of 26 such malnourished infants

associated with other acute diseases such as pneumonia, gastroenteritis etc. The degree of malnutrition was estimated according to a modification of the criteria of Gomez *et al* for Mexican children (14). There were 26 patients (13 males and 13 females) whose ages varied from nine months to three years. Four of these patients had first degree malnutrition, ten had second degree, seven had third degree and five fourth degree. A detailed history was obtained for each one with special emphasis on his nutritional state. Usual methods were used for the haematological tests (7). In some patients serum iron, unsaturated iron binding capacity in the serum and serum folic acid levels were measured (17, 20, 29). Serum proteins were measured by the biuret method (15). D-Xylose absorption tests were done on fourteen patients and oral glucose tolerance tests on thirteen by using standard described methods (18, 22, 34). The glucose tolerance curve is considered normal when a 30 mg increase over the fasting level in 30 min and a return to the original level within two hours. In a diabetic curve the blood sugar increases to over 160 mg and does not return to the original level within two hours.

Jejunal biopsies were performed by a technique previously described elsewhere using a paediatric size (8 mm x 17 mm) Crosby capsule (2). The specimen was examined under a hand loop before being pinned out on a card, mucosal surface uppermost and fixed in a 10 per cent buffered formalin. No examinations were done under the dissecting microscope. After fixation the specimens were imbedded in paraffin wax and sections were cut and stained with haematoxylin and eosin and by the periodic acid-Schiff technique. Care was taken to prevent tangential cuts of the specimen. None of the infants were acutely ill or suffering from gastroenteritis at the time of biopsy.

In the histologic examination of the mucosa: 1) height of the villi; 2) epithelial cells of the villi; 3) depth of the crypts; and 4) cellularity and/or oedema of the lamina propria were carefully checked. In normal jejunal mucosa the villi are finger-like or long; the epithelial cells are columnar with basally and regularly arranged nuclei and existing microvilli.

## MATERIAL AND METHODS

The patients were all seen at Hacettepe Children's Hospital in Ankara, Turkey, between September 1965 and September 1966. They all had malnutrition as

This work was supported by a grant from Türkiye Bilimsel ve Teknik Araştırma Kurumu, Ankara, Türkiye (Scientific and Technical Research Council of Turkey).

*1 Clinical and haematological data*

Age (months) and sex	Weight (kg) Degree of malnutrition	Serum proteins (g/100 ml) alb/glob	Haemoglobin (g/100 ml)	Serum iron Serum iron binding Cap (γ/100 ml)
30/♂	6.3/IV	$78 = \frac{53}{25}$	6.75	—
36/♂	6.8/IV	$63 = \frac{38}{28}$	6.65	40/333
30/♀	6.71/IV	$65 = \frac{44}{21}$	8.45	—
18/♀	5.5/IV	$72 = \frac{46}{26}$	3.0	136/312.5
24/♂	5.83/IV	—	6.95	—
18/♀	6.38/III	$64 = \frac{42}{22}$	8.45	80/390
18/♂	6.7/III	$46 = \frac{15}{31}$	7.40	—
27	10.5/I	$60 = \frac{38}{22}$	10.80	—
18/♀	6.24/III	$42 = \frac{22}{20}$	7.17	90/390
25	10.8/—	$68 = \frac{42}{26}$	11.55	—
36/♀	8.5/III	$64 = \frac{44}{20}$	3.85	28/314
42	10/—	$56 = \frac{30}{26}$	12.30	41/417
9/♂	4.8/III	$34 = \frac{18}{16}$	9.20	—
10/♂	5.9/III	$61 = \frac{39}{22}$	10.05	—
14/♂	5.8/III	—	9.20	—
14/♂	7.5/II	$7 = \frac{55}{15}$	9.20	67/334
19.5	10.1/I	—	10.80	—
30	10.3/II	$45 = \frac{25}{20}$	6.95	64/350
12/♂	6.3/II	$75 = \frac{55}{20}$	9.50	—
30 ♀	8.7/II	$65 = \frac{45}{20}$	11.55	—
24/	7.5/II	$68 = \frac{41}{27}$	11.40	—
18/♂	8.1/II	$68 = \frac{38}{30}$	12.30	87/390
12	6.5/II	$66 = \frac{42}{24}$	9.6	95/346
18.5	7.8/II	$68 = \frac{38}{30}$	11.25	—
36	9.9/II	$48 = \frac{28}{20}$	5.45	42/408
40	11.5/I	$62 = \frac{40}{22}$	10.05	—
20	8.7/II	$62 = \frac{40}{22}$	8.30	183/248
4.5	9.3/I	$42 = \frac{22}{20}$	6.95	157/350
27	13/—	$70 = \frac{45}{25}$	11.85	—
36	12/I	$62 = \frac{37}{25}$	9.65	49/330

Table 1 (cont.)

Case no	Age (months) and sex	Weight (kg) Degree of malnutrition	Serum proteins (g/100 ml) alb/glob	Haemoglobin (g/100 ml)	Serum iron Serum iron binding Cap (γ/100 ml)
	44	15/—	6.5 = $\frac{3.8}{2.7}$	8.45	—
25	24/♀	10.0%	4.5 = $\frac{2.6}{1.9}$	8.90	—
	31	12.2/—	6.2 = $\frac{3.4}{2.8}$	8.00	48/364
26	10/♀	6.13/1	—	6.80	—

Second admission — = Not done (—) = negative

the crypts show no increase in depth and the lamina propria exhibits no oedema or hypercellularity. Deviations from these characteristics are considered to be abnormal stemming from a mild to severe degree of atrophy. Slight broadening or branching of the villi with areas of cuboidal cells and some degree of atrophy increased depth of crypts slight increase in the cellular infiltration of the lamina propria indicate mild histologic changes. Flat or short villi with atrophy in the epithelium increase in the cellular infiltration of the lamina propria are considered as severe changes. The terms of partial or subtotal villous atrophy were not used in this study as it has been claimed that they do not properly describe histologic changes (23). Each section was assessed at least twice with consistent results. Disaccharidase activities were determined by a micro method (9) which is a modification of Dahlquist's technique (13). For this purpose the biopsy specimen was cut into two pieces: the first half was frozen in dry ice immediately and was kept at  $-20^{\circ}\text{C}$  in the freezer until the assay was performed. The second half was fixed in buffered formalin for histological interpretation. At the time of assay the frozen biopsy specimen was homogenized in a stirred walled all glass homogenizer. The protein content was determined by the Warburg

method (9). The estimation of invertase, lactase and maltase was carried out on a microscale by estimating the glucose liberated from these disaccharides by the hydrolases. For this purpose a purified yeast hexokinase and glucose 6-phosphate dehydrogenase coupled system was used (8). Biopsy specimens weighing as little as 0.07–0.1 mg were enough to obtain a satisfactory answer to the presence of hydrolase in the mucosa. The activities were expressed in units as micromoles of disaccharide hydrolyzed per milligram of protein content of biopsy specimen per hour. The assay was conducted at room temperature which varied between 25–27°C. Normal values in our laboratory for lactase, maltase and invertase are 0.112 (0.050–0.130) units/hr/mg protein, 0.799 (0.250–0.340) units/hr/mg protein, 1.666 (0.130–0.190) units/hr/mg protein respectively.

Seven children without malnutrition between the ages of 12 and 36 months formed a control group.

## RESULTS

The age, sex, weight, degree of malnutrition and the laboratory findings are summarized in



Fig. 1. Appearance of the jejunal mucosa in an 18-month-old child with third degree of malnutrition (case 7). Note that villi are broad with areas of atrophy in epithelium. The Lieberkuhn crypts are elongated. Lamina propria contains increased number of mononuclear cells (lymphocytes, plasma cells and some eosinophils) and exhibits some mild oedema.

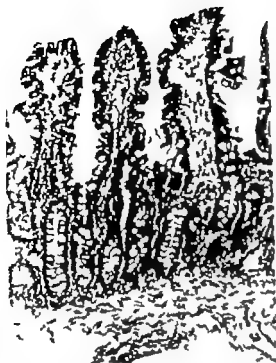


Fig. 2. Repeat biopsy of the same case 9 months after therapy. The villi appear finger-like; the epithelial cells are columnar. Lamina propria exhibits no oedema or hypercellularity.

Table 1. Seven of the 23 patients whose serum protein levels were measured had total protein levels of 4.8 g/100 ml or less. Only five patients had albumin values below 2.5 g/100 ml. Their haemoglobin values varied from 3 g to

12.30 g/100 ml. All but four were anaemic with haemoglobin levels under 10.50 g/100 ml. After treatment these values improved rapidly. In fifteen cases bone marrow showed normoblastic erythroid hyperplasia. Serum iron was less than 70 µg/100 ml with a high iron binding capacity in eight of fourteen patients on whom the test was performed. Serum folic acid levels were determined for eleven patients and all were found to be normal. Traces of occult blood were found in the stool of five patients and two patients stool examinations revealed ascariasis. The glucose tolerance was flat in one (case 24) of the twelve patients on whom it was performed; another patient (case 15) had a diabetic curve. The D-Xylose absorption test was normal in all of the thirteen patients tested. A total of 34 jejunal biopsies were taken including the eight repeat biopsies. No major biopsy complications such as diffuse haemorrhage or perforation occurred. In twelve patients occult blood was positive for 24–48 hours after the biopsy. Seventeen specimens showed mild abnormalities in the mucosa characterized by broader and shorter villi with a tendency to branch; areas of cuboidal cells and some degree of atrophy; increased depth of crypts; increased cellular infiltration (consisting of lymphocytes, eosinophils and plasma cells) and oedema of the lamina propria (Fig. 1). The mucosa in seven patients was normal. Two specimens were considered insufficient for histologic evaluation.

Table 2. Absorption tests, disaccharidase levels and histology.

Case no.	Degree of malnutrition	Absorption tests		Disaccharidase levels			Histology of the jejunal mucosa
		Glucose tol.	D-Xylose	Lactase	Maltase	Invertase	
3	IV	N	N	0.000	0.030	0.030	Ab
15	IV	—	N	0.000	0.007	0.030	Insufficient
16	II	Diabetic	N	0.002	0.010	0.010	Ab
17	II	—	N	0.052	0.010	0.070	N
18	II	N	—	0.074	0.060	0.050	Ab
19	II	N	—	0.000	0.070	0.020	Insufficient
20	II	N	—	0.021	0.090	0.058	Ab
21	II	N	N	0.000	0.080	0.072	Ab
21	I	—	—	0.003	0.340	0.016	N
		—	—	—	—	—	N

Second admission. Ab=Abnormal. N=normal.

Table 1 (cont.)

Case no	Age (months) and sex	Weight (kg) Degree of malnutrition	Serum proteins (g/100 ml) alb/glob	Haemoglobin (g/100 ml)	Serum iron Serum iron binding Cap (γ/100 ml)
	44	15/—	65 = $\frac{38}{27}$	8.45	—
25	24/♀	10.0/I	45 = $\frac{26}{19}$	8.90	—
	31	12.2/—	62 = $\frac{34}{28}$	8.00	48/164
26	10/♀	6.33/I	—	6.80	—

Second admission — = Not done (—) = negative

the crypts show no increase in depth and the lamina propria exhibits no oedema or hypercellularity. Deviations from these characteristics are considered to be abnormal stemming from a mild to severe degree of alteration. Slight broadening or branching of the villi with areas of cuboidal cells and some degree of atrophy increased depth of crypts slight increase in the cellular infiltration of the lamina propria indicate mild histologic changes. Flat or short villi with atrophy in the epithelium increase in the cellular infiltration of the lamina propria are considered as severe changes. The terms of partial or subtotal villous atrophy were not used in this study as it has been claimed that they do not properly describe histologic changes (23). Each section was assessed at least twice with consistent results. Disaccharidase activities were determined by a micro method (8) which is a modification of Dahlquist's technique (13). For this purpose the biopsy specimen was cut into two pieces: the first half was frozen in dry ice immediately and was kept at  $-20^{\circ}\text{C}$  in the freezer until the assay was performed. The second half was fixed in buffered formalin for histological interpretation. At the time of assay the frozen biopsy specimen was homogenized in a suntered walled all glass homogenizer. The protein content was determined by the Warburg

method (9). The estimation of invertase, lactase and maltase was carried out on a microscale by estimating the glucose liberated from these disaccharides by the hydrolases. For this purpose a purified yeast hexokinase and glucose 6 phosphate, dehydrogenase coupled system was used (8). Biopsy specimens weighing as little as 0.07–0.1 mg were enough to obtain a satisfactory answer to the presence of hydrolase in the mucosa. The activities were expressed in units as micromoles of disaccharide hydrolyzed per milligram of protein content of biopsy specimen per hour. The assay was conducted at room temperature which varied between  $25\text{--}27^{\circ}\text{C}$ . Normal values in our laboratory for lactase, maltase and invertase are 0.112 (0.090–0.130) units/hr/mg protein, 0.295 (0.250–0.340) units/hr/mg protein, 0.166 (0.130–0.190) units/hr/mg protein respectively.

Seven children without malnutrition between the ages of 12 and 36 months formed a control group.

## RESULTS

The age, sex, weight, degree of malnutrition and the laboratory findings are summarized in



Fig. 1. Appearance of the jejunal mucosa in a 18 month old child with third degree of malnutrition (case 7). Note that villi are broad with areas of atrophy in epithelium. The lamina propria contains increased number of mononuclear cells (lymphocytes, plasma cells and some eosinophils) and exhibits some mild oedema.



Repeat biopsy specimens showed that the appearance of the mucosa returned to normal in 4 patients (cases 7, 13 and 24) after correction of their anaemia and malnutrition (Fig. 2). In these patients the intervals between the first and second biopsy were 9.7, 5.5 and 8 months respectively. Cases 9, 21, 23 who initially had normal mucosae also showed normal histology in repeat biopsies performed 6.4 and 3.5 months after the first one. In one patient (case 25) the abnormal histology persisted and could be seen in the second biopsy which was done seven months later. At this time, the malnutrition was corrected but the patient was still anaemic.

Disaccharidase assays were performed in nine patients (Table 2). All had decreased activities of lactase, maltase and invertase compared to normal controls. The different enzyme activity levels varied as follows: Lactase from 0.000 to 0.052 (mean 0.011 unit), maltase from 0.007 to 0.340 (mean 0.071 units) and invertase from 0.010 to 0.072 (mean 0.039 units).

The absorption tests and jejunal biopsies on seven control cases revealed no abnormalities.

## DISCUSSION

The results of this study indicate that the jejunal mucosa in malnourished children exhibits some mild histologic changes accompanied by a decrease in disaccharidase activities. Histologic changes seen in our cases were similar to those found by Stanfield *et al* (31) and Burman (6) in African children with kwashiorkor. Sprinz and his co-workers also described the same changes in Thai people from a low socioeconomic background (27). Tropical sprue (1), hookworm anaemia and iron deficiency anaemia in infants (19) and children (3, 16) may cause similar histologic changes in the intestinal mucosa, the most severe is being described in coeliac sprue (4, 27). Brunser and co-workers (5) reported mild histologic changes in four and more marked changes in one of the 18 marasmic infants studied.

In this series 6 of the ten infants with kwashiorkor exhibited a completely flat mucosa, another 3 had flat areas with a few short villi.

Stanfield *et al* (31) noted the persistence of mucosal changes in follow up studies one year after treatment for kwashiorkor. In their series, there was rapid clinical improvement and complete biochemical recovery but the jejunal mucosa remained abnormal. Cook & Lee (10) studied African children who had had kwashiorkor four years or more previously and follow up biopsies in 18 of these children revealed that microscopic alterations in jejunal mucosa converted to normal in most of them.

In our repeat biopsies it was observed that these non specific mucosal alterations were reversible. However in a 24 month old girl with first degree malnutrition (case 25) mucosa still showed the same abnormalities 7 months after her initial examination at which time she was not malnourished but had iron deficiency anaemia.

There was no correlation between the jejunal mucosal changes and anaemia, hypoproteinaemia or severity of malnutrition. There was also no parallelism between the results of absorption tests and intestinal histology.

Multiple factors such as deficiencies of proteins, iron and folic acid may possibly respond to the above mentioned alterations in jejunal mucosa. Protein is the main substance in the structure of the enzymes which are essential for the function and integrity of epithelial cells. Nauman *et al* (19) described histologic changes similar to those seen in malabsorption syndromes in the duodenal mucosa of infants with iron deficiency anaemia. After iron treatment, the mucosa became normal in these infants and children. Recently Guha *et al* (16) demonstrated the presence of mild to severe histological abnormalities in children with iron deficiency anaemia resembling those seen in malabsorption states. Patients with folic acid deficiency often develop diarrhoea and this is also reported in cases of tropical sprue with typical mucosal changes and malabsorption (1).

However protein iron and/or folic acid deficiency may not be the only factors influencing the intestinal mucosa. There may also be some mucosal trophic factors or a specific trace mineral factor present in the diet (11). Addition of these missing factors to the patient's diet will generally correct the condition of the mucosa. It is claimed that an environment relatively deficient in protein could enable the mucosa gradually to return to normal (11). Mucosal changes may however be irreversible as observed in adults of low socio-economic groups in Thailand and among normal adults in Uganda and Southern India (14, 30). After exclusion of a genetic basis for these changes it has been suggested that the persistence of mucosal change in adulthood may be the end result of a long standing chronic low protein diet in infancy or childhood (31).

In this study lactase maltase and invertase in the jejunal mucosa of nine patients were markedly decreased in comparison with normal controls. The levels were at least 3.5-4 times reduced in all enzymes. Here the disaccharidase deficiency might be related to malnutrition and alteration in the jejunal epithelium. Stanfield *et al* (31) found low disaccharidase levels in children with kwashiorkor but with remission of the disease enzyme levels may improve remarkably (10). We had no repeat disaccharidase assays done after the initial ones in the nine infants of this series. With older malnourished children our experience was similar to that of Stanfield *et al* (31) and Cook & Lee (10). Absorption tests, intestinal biopsies and disaccharidase assays were carried out in four children aged 4 to 6 years. Two had second degree and the other two had fourth degree malnutrition. They had normal glucose tolerance curves. All had decreased lactase maltase and invertase activities ranging from 0.000 to 0.030, 0.096 to 0.190, 0.040 to 0.090 units respectively. Patients with fourth degree malnutrition showed abnormal jejunal histology which was still present 7 and 9 months after initial examination at which time they were less malnourished. Disaccharidase assays

showed some improvement in one. Only invertase activity was found to be increasing in the other.

It is reported that lactase is more severely depressed than other disaccharidases and is slow to recover in coeliac sprue and tropical sprue after treatment (21, 25, 28). We also found that lactase levels were the lowest in our 9 marasmic infants. However the ratios of lactase to maltase and lactase to invertase were constant as expected in secondary disaccharidase deficiencies (31, 32). The return of lactase activity to normal may be slower in malnourished children who exhibit a high degree of lactase intolerance. Finally it should be mentioned that these secondary deficiencies may very well be the cause of chronic diarrhoeas and food intolerance in many marasmic children.

## SUMMARY

Twenty six infants with malnutrition and seven controls were studied by routine haematological tests, serum protein levels, glucose tolerance and D-Xylose absorption tests, jejunal biopsies and intestinal disaccharidase assays. In conclusion it can be said that in malnutrition the jejunal mucosa shows some mild or non-specific histologic changes compatible with malabsorption which may be reversible. No correlation could be shown between these changes and absorption tests. Malnutrition may also result in secondary intestinal disaccharidase deficiencies which improve with correction of the nutritional state. These secondary enzyme deficiencies may be responsible for some of the diarrhoeas seen in marasmic children. No direct correlation between anaemia, iron-deficiency, folic acid levels, protein deficiency and intestinal mucosal changes could be established in this study.

## ACKNOWLEDGEMENT

We are indebted to Dr Pinar Özand and his colleagues for disaccharidase determination on biopsy specimens.



## REFERENCES

- Baker S J, Ignatius M, Myrhan V J, Varsh S A & Charko C C. Intestinal biopsy in tropical sprue. *Ciba Symposium Study Group in Intestinal Biopsy*. Basle 1962 p 84
- Berkel I & Kuran O. Importance of the per oral intestinal biopsy in children as a diagnostic aid (in Turkish). *Paper presented at the IVth Seminar of the Turkish Ped Soc July 1964 Istanbul*
- Berkel I, Say B & Kuran O. Intestinal mucosa in children with pica and iron deficiency anaemia (in preparation)
- Booth C C, Stewart J S, Holmes R & Brick enbury W. Dissecting microscope appearance of intestinal mucosa. *Intestinal Biopsy Ciba Found Study Group 14 J A Churchill Ltd London 1962 p 2*
- Brumer O, Reid A, Monclberg F, Muccioni A & Contreras I. Jejunal biopsies in infant mal nutrition with special reference to mitotic index. *Pediatrics* 39 605 1966
- Burman D. The jejunal mucosa in kwashiorkor. *Arch Dis Child* 40 526 1965
- Cartwright G E. *Diagnostic laboratory hematology*. Grune and Stratton Inc. New York 1958 2nd ed p 34
- Ciliv G & Özand P. Measurement of invertase, maltase and beta galactosidase activities on small samples of intestinal mucosa. *Turkish J Ped* 7 204 1965
- Colowick S P & Kaplan N O. *Methods in enzymology*. Vol 3. Acad Press Inc. New York 1957 vol 3 p 451
- Cook G C & Lee F D. The jejunum after kwashiorkor. *Lancet* II 1263 1966
- Creamer B. Small intestinal mucosal dynamics and environment. *Brit Med J* II 1373 1964
- Crosby W H & Kugler H W. Intraluminal biopsy of the small intestine: the intestinal biopsy capsule. *Am J Digest Dis* 2 236 1957
- Dahlquist A. Method of assay of intestinal disaccharidases. *Anal Biochem* 7 118 1964
- Dogramaci I & Wray J. Severe infantile mal nutrition and its management. *Turkish J Ped* 1 129 1958
- Fisher H J. *Manual of standardized procedures for spectrophotometric chemistry*. Sci Supply Corp. Pub. New York. P A 10(1-3) G 15(1-3) and P 50(1-6) 1950
- Guha D K, Wila B N S, Tandon B N, Deo M G & Ghai O P. Small bowel changes in iron deficiency anaemia of childhood. *Arch Dis Child* 43 239 1968
- Herbert V, Fisher R & Koonitz B J. The assay and nature of folic acid activity in human serum. *J Clin Invest* 40 81 1961
- Meites S & Faulkner W S. *Manual of practical and general procedures in clinical chemistry*. C C Thomas Pub. Springfield Ill 1962 p 231
- Nauman J L, Oski F A, Diamond L K, Vawter G F & Schwachman H. The gastrointestinal effects of iron-deficiency anemia. *Pediatrics* 33 83 1964
- Peters T, Giovannelli T J, Apt L & Ross J F. A new method for the determination of serum iron binding capacity. *J Lab Clin Med* 48 274 1956
- Plotkin G R & Isselbacher K J. Secondary disaccharidase deficiency in adult celiac disease (non tropical sprue) and other malabsorption states. *New Eng J Med* 271 1033 1964
- Rapoport M. Glucose tolerance test (oral). In Nelson (ed) *Textbook of Pediatrics*. W B Saunders Co Philadelphia 1959 7th ed p 698
- Rubin C E, Dobbin W D. Peroral biopsy of the small intestine. A review of its diagnostic usefulness. *Gastroenterology* 49 676 1965
- Scrimshaw N S & Behar M. Malnutrition in underdeveloped countries. *New Eng J Med* 277 137 1965
- Sheehy T W, Anderson P R & Bress H E. Carbohydrate studies in tropical sprue. *J Indian Med Prof* 12 4432 1965
- Shiner M. Duodenal biopsy. *Lancet* I 17 1946
- Shiner M & Doulich I. Histopathologic studies in steatorrhea. *Gastroenterology* 38 419 1960
- Shmerling D H, Aumecho S, Rubin A, Hadorn H & Prader A. Der sekundäre Mangel an intestinaler Disaccharidaseaktivität bei der Coliase. *Helvet Paed Acta* 19 507 1964
- Simmonds J S & Gentzkow C J. Determination of serum iron. *Med Pub Health Vet Lea and Fetscher Philadelphia* 1955 p 417
- Sprinz H, Sribhubbadi R, Gangarosa E J, Benayahu C, Hundel D & Halstead S. Biopsy of small bowel of Thai people with special reference to recovery from Asiatic cholera and to its intestinal malabsorption syndrome. *Amer J Clin Path* 38 43 1962
- Stanfield J P, Hutt M S & Tunnicliffe R. Intestinal biopsy in kwashiorkor. *Lancet* II 519 1965
- Townley R W. Disaccharidase deficiency in infancy and childhood. *Pediatrics* 38 127 1966
- Trowell H C, Davies J N P & Dean R F. A. *Kwashiorkor*. Edward Arnold and Co. London 1954 p 149
- Varley H. Blood sugar and its determination: method of Folin and Wu. *Pract Clin Biochem*. William Heineman Med Books Ltd London 1958 2nd ed p 33

Submitted April 22 1969

Accepted July 4 1969

II B) Hacettepe Childrens Hospital  
Ankara  
Turkey

Key words: Infants malnutrition intestinal biopsy disaccharidases

## CHROMOSOMAL CONDITIONS IN CONGENITAL HEART DISEASE

GUDRUN DAHL

From Queen Louise's Children's Hospital Copenhagen Denmark

Congenital cardiovascular disease is often found as an associated malformation in patients with various chromosomal abnormalities. It is thus a well known observation that in patients with Down's syndrome, Turner's syndrome, cri du chat, trisomy D and trisomy E congenital heart disease shows a far greater incidence than in normal subjects. However, congenital heart disease also occurs frequently together with other malformations in patients showing more unusual chromosomal abnormalities such as partial trisomy in groups A and C.

Chromosomal abnormalities in a mother and son who both had a congenital heart disease—atrial septal defect—as sole malformation (2) were reported for the first time in 1961. Since then several chromosomal studies have been published in a large number of patients with congenital heart disease with or without other accompanying malformations. Thus, Sakaki *et al.* (13) examined the chromosomal picture in 22 patients with congenital heart disease. Three patients presented a clinical picture of Down's syndrome with trisomy G. Eleven patients were cytogenetically normal. In 9 patients (one of whom also had trisomy G) chromosome no. 16 showed differences in size presenting a picture of monosomy for no. 16 and trisomy for no. 19. Such a heterozygous

condition for pair no. 16 varied in degree even in the same patient.

Anders *et al.* (1) examined 156 patients with congenital heart disease, patients with Down's syndrome and Turner's syndrome being excluded from the material. Among these 156 patients none were found with major chromosomal abnormalities. Five patients, however, showed minor variations in the size of individual chromosomes. For example, in 2 patients one chromosome no. 16 was as large as no. 12. The increased length was apparently due to the elongation of the secondary constriction normally found in the paracentric region of the long arm. In another 2 patients the majority of the cells showed one 16 shorter than usual and in extreme instances it resembled a 19. In the fifth patient the short arms on a chromosome 15 were unusually long. Family members were examined in 2 of the cases and some of them showed the same chromosomal abnormalities as the probands although not resulting in congenital heart disease.

In 1966 Rohde (11) published 68 cases with heart disease where the karyotype had been studied. The author had been particularly interested in patients with accompanying congenital malformations, ovarian and testicular dysgenesis and certain mental, atrophic and neurological disorders. Eleven patients were representative of the clinical syndromes associated with simple or partial autosomal trisomy in groups C, D, E and G and 10 patients in

This investigation was made possible by grants from Forenspet og Hætereydonomes Næstingstiftelse, F. L. Schmidt & Co. A/S's Jubilæumsfond and Statens Almindelige Væsenstilsel.

## REFERENCES

- 1 Baker S J Ignatus M Mathan V I Vash S K & Charko C C Intestinal biopsy in tropical sprue *Ciba Symposium Study Group in Intestinal Biopsy* Basle 1962 p 84
- 2 Berkel I & Kiran O Importance of the per oral intestinal biopsy in children as a diagnostic aid (in Turkish) *Paper presented at the IVth Seminar of the Turkish Ped Soc July 1964 Istanbul*
- 3 Berkel I Say B & Kiran O Intestinal mucosa in children with pica and iron deficiency anaemia (in preparation)
- 4 Booth C C Stewart J S Holmes R & Brackenbury W *Dissecting microscope appearance of intestinal mucosa Intestinal Biopsy Ciba Found Study Group 14 J A Churchill Ltd London 1962 p 2*
- 5 Brunser O Reid A Monckeborg F Macconni A & Contreras I Jejunal biopsies in infant malnutrition with special reference to mitotic index *Pediatrics* 38 605 1966
- 6 Burman D The jejunal mucosa in kwashiorkor *Arch Dis Child* 40 526 1965
- 7 Cartwright G E *Diagnostic laboratory hematology* Grune and Stratton Inc New York 1958 2nd ed p 34
- 8 Ciliv G & Özand P Measurement of invertase, maltase and beta galactosidase activities on small samples of intestinal mucosa *Turkish J Ped* 7 204 1965
- 9 Colowick S P & Kaplan N O *Methods in enzymology* Vol 3 Acid Press Inc New York 1957 vol 3 p 451
- 10 Cook G C & Lee F D The jejunum after kwashiorkor *Lancet* II 1263 1966
- 11 Cramer H Small intestinal mucosal dynamics and environment *Brit Med J* II 1373 1964
- 12 Crosby W H & Kugler H W Intraluminal biopsy of the small intestine: the intestinal biopsy capsule *Am J Digest Dis* 2 236 1957
- 13 Dahlquist A Method of assay of intestinal disaccharidases *Anal Biochem* 7 18 1964
- 14 Dogramaci I & Wray J Severe infantile malnutrition and its management *Turkish J Ped* 1 129 1958
- 15 Fisher H J *Manual of standardized procedures for spectrophotometric chemistry* Sci Supply Corp Pub New York P A 10(1-3) G 15(1-3) and P 50(1-6) 1950
- 16 Guha D K, Walia B N S, Tandon B N, Deo M G & Ghai O P Small bowel changes in iron deficiency anaemia of childhood *Arch Dis Child* 43 239 1968
- 17 Herbert V, Fisher R & Koonitz B J The assay and nature of folic acid activity in human serum *J Clin Invest* 40 81 1961
- 18 Meites S & Faulkner W S *Manual of practical and general procedures in clinical chemistry* C C Thomas Pub Springfield Ill 1962 p 231
- 19 Nauman J L, Oaks F A, Diamond L K, Vawter G F & Schwachman H The gastro intestinal effects of iron deficiency anemia *Pediatrics* 33 83 1964
- 20 Peters T, Giovannello T J, Apt L & Ross J F A new method for the determination of serum iron binding capacity *J Lab Clin Med* 48 274 1956
- 21 Plotkin G R & Isselbacher K J Secondary disaccharidase deficiency in adult celiac disease (non tropical sprue) and other malabsorption states *New Eng J Med* 271 1033 1964
- 22 Rapoport M Glucose tolerance test (oral) in Nelson (ed) *Textbook of Pediatrics* W B Saunders Co Philadelphia 1959 7th ed p 698
- 23 Rubin C E, Dobbins W D Peroral biopsy of the small intestine A review of its diagnostic usefulness *Gastroenterology* 49 676 1965
- 24 Scrimshaw N S & Behar M Malnutrition in underdeveloped countries *New Eng J Med* 272 137 1965
- 25 Sheehy T W, Anderson P R & Bangs B E Carbohydrate studies in tropical sprue *J Indian Med Prof* 12 5432 1965
- 26 Shiner M Duodenal biopsy *Lancet* I 17 1956
- 27 Shiner M & Doanach I Histopathologic studies in steatorrhea *Gastroenterology* 38 419 1960
- 28 Shmerling D H, Auncsoo S, Rubino A, Hadorn B & Prader A Der sekundäre Man El in intestinaler Disaccharidaseaktivität bei der Colik *Helvet Paed Acta* 19 507 1964
- 29 Simmonds J S & Gentzkow C J Determination of serum iron *Med Pub Health Met Lea and Febriger* Philadelphia 1955 p 417
- 30 Sprinz H, Scribbsbath R, Gangarosa E J, Benayahu C, Kundel D & Halstead S Biopsy of small bowel of Thai people with special reference to recovery from Asiatic cholera and to an intestinal malabsorption syndrome *Amer J Clin Path* 38 43 1962
- 31 Srinfield J P, Hatt M S & Tunnicliffe R Intestinal biopsy in kwashiorkor *Lancet* II 519 1965
- 32 Townley R W Disaccharidase deficiency in infancy and childhood *Pediatrics* 38 127 1966
- 33 Trowell H C, Davies J N P & Dean R F A *Kwashiorkor* Edward Arnold and Co London 1954 p 149
- 34 Varley H Blood sugar and its determination method of Folin and Wu *Pract Clin Biochem* William Heineman Med Books Ltd London 1958 2nd ed p 33

Submitted April 22, 1969

Accepted July 4, 1969

(I B) Hacettepe Childrens Hospital  
Ankara  
Turkey**Key words** Infants malnutrition intestinal biopsy disaccharidases

Table 1 Age sex chromatin and distribution of chromosome counts for 82 patients with congenital cardiovascular disease

Ref no	Age (yrs)	Sex chromatin (positive)	Chromosome counts					Total cells
			<45	45	46	47	>47	
12	4	3		1	28	1		30
13	1 5/12	2		2	48			50
19	3	27	1	5	38		1	43
4	5/12	40	2	3	34	1		40
37	11/12	5	3	8	25			36
41	2	45	3	2	28	1		34
42	1	9	4	1	38			43
44	1	9	1	2	38			41
45	5	5	1	2	31			34
49	6/12	3		3	36	1		40
54	4/12	2	5	3	33			41
55	1/12	31	1	3	37			41
57	1/12	6	2	5	32			39
59	6	34	1	5	35			41
61	1	3	3	6	36			45
66	0/12	2	1	1	34			36
67	1/12	1			38	1		39
68	8/12	1	7	5	40			57
74	3/12	52	13	4	41			58
75	4/12	5	1	5	36			40
81	1/12	5	4	4	42	1		51
87	0/12	8	3	2	30			35
89	4	36		3	37			40
91	0/12	31	1	4	38			43
92	2	33		3	37			40
93	1	1	2	7	37			46
94	10/12	32	2	5	51	2		60
103	2/12	33	1	2	28			31
104	1	30	2	3	36			41
105	3/12	1	5	11	34			30
108	3	40	6	3	41			50
109	2	27	3	2	35			40
110	0/12	4	2	2	46			50
112	10/12	25	1	2	38			41
117	2/12	28		1	40			41
119	9/12	31			30			30
123	0/12	30	2	6	32			40
127	2	51	1	3	42			46
129	1/12	1	3	3	44			50
134	10/12	36	1	5	44			50
137	4	44	2	1	45			46
138	3/12	38	4	4	44			50
139	5	3	1	6	36			46
140	3/12	4	4	2	47			50
143	3/12		2	5	37			46
144	2	24			48			50
145	5/12	4	1	3	37			40
146	1	5		12	60			73
147	3/12	5		2	38	1		41
149	5/12	74		1	49			50
155	1	3	3	3	40			43
158	11	4	1	1	41			43
159	10	40	2	5	33			41
161	7	1	2	4	32	1		39
163	6	31	1	2	37			41
164	1	1	2	4	36			42
166	10	1	1	3	36			40
171	6	38	4	3	50			57
177	5/12	3	1	1	49			50
183	2			5	47			48

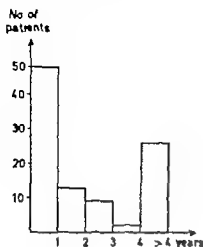


Fig 1 The age distribution

abnormalities of the sex chromosomes. In 34 patients with congenital heart disease and in 13 patients with cardiovascular disorders, normal chromosomal conditions were found.

Emerit *et al* (6) examined 275 patients with congenital heart disease, selected either for the presence of extracardiac abnormalities or the existence of congenital heart disease in other members of the family. Thirty nine patients had classical chromosomal abnormalities. Sixteen other patients had minor autosomal abnormalities. In numerous cases these abnormalities were found in only a few of the cells examined. The karyotypes of a few of the family members were also examined. In one patient, for example, 10 out of 14 cells showed a small acentric chromosome fragment which was not found in the mother. In another patient and his mother a constant break was found in the long arm of a group C chromosome. In 2 brothers both with congenital heart disease, a chromosome in the C group was found to be abnormally large and in many of the cells this chromosome showed a secondary constriction. The same abnormality was also found in the mother and in another brother although they did not show any congenital malformations.

## MATERIAL AND METHODS

Many children are admitted annually to Queen Louise's Childrens Hospital for examination and

treatment for congenital cardiovascular disease. It was therefore possible to carry out an extensive investigation as to whether any relationship could be demonstrated between chromosomal abnormalities and congenital cardiovascular disease—and in particular whether there were variations in the size of the chromosomes also in this material described in some of the studies mentioned above.

The investigation took place during the years 1964–67 the material consisting of 100 patients in whom the diagnosis of cardiovascular disease was confirmed by cardiac catheterization, angiocardiotherapy or operation. In some cases of persistent ductus arteriosus where the diagnosis was so obvious that cardiac catheterization was unnecessary the diagnosis was verified by operation alone. The material includes practically all forms of congenital heart defect and patients who also had other malformations or known syndromes were not excluded from the material.

The karyotype was studied on blood cultures prepared according to Erlenfeld (7) a microtechnique based on the principles of Moorhead *et al* (1960). Nuclear sex was determined on buccal smears stained by Feulgen method. In our laboratory about 200 sex chromatin determinations were made in patients without sex chromosome abnormalities. In this material less than 9% of the cells in the buccal smear in the case of phenotypic boys were found to contain a chromatin body (=sex chromatin negative) while in the case of phenotypic girls more than 19% of the cells were found to contain a chromatin body (=sex chromatin positive).

## RESULTS

Fifty four out of the 100 patients were phenotypic girls. Of these 50 were sex chromatin positive, while 4 were sex chromatin negative. These 4 showed clinical signs of Turner's syndrome with a karyotype 45/X0. The remaining 46 patients were phenotypic boys. 45 of whom were sex chromatin negative. (In 1 patient (no 142) the sex chromatin determination failed and the child died before a new investigation could be made.)

Fig 1 shows the age distribution. Half of the patients were less than 12 months old at the time of investigation and 39 were even less than 6 months old.

In 82 out of the 100 patients no major chromosome abnormalities were found. The chromosome investigations showed a modal number of 46 and the analysis revealed a normal karyotype for the sex. Age nuclear sex

Table 3 Age sex chromatin and distribution of chromosome counts in 4 patients with congenital cardiovascular disease and Turner's syndrome

Ref no	Age (yrs)	Sex chromatin (positive)	Chromosome counts					Total cells
			<45	45	46	47	>47	
16	1	4	1	50				57
249	17	1	6	35				41
270	12	0	2	49				51
304	2	4	1	39				40

Table 4 Age sex chromatin and distribution of chromosome counts in 2 patients with congenital heart disease and chromosome abnormalities

Ref no	Age (yrs)	Sex chromatin (positive)	Chromosome counts					Total cells
			<45	45	46	47	>47	
123	3/12	6	2	2	43			49
189	1/12	1	5	5	46	1		57

negative with a karyotype 45/X0 without mosaicism (Table 3)

Two patients on the other hand had unusual karyotypes (Table 4) for which reason they will be described in more detail. The first patient (no 123) was a 3 month old boy. An elder brother as well as both parents were healthy and phenotypically normal. The parents were unrelated and there were no malformations in the family. The father was 27 years old and the mother 25 years old when the patient was born. The mother had never

aborted. Pregnancy and delivery were normal. Birth weight was 3500 g and birth length 54 cm. After birth contractures of the finger were observed as well as a single crease on either little finger. From birth there was occasionally dyspnoea and later attacks of cyanosis and apnoea. A roentgenogram of the thorax, cardiac catheterization and operation showed retro-aesophageal vessels, patent ductus arteriosus, anomalies of pulmonary venous return and right aortic arch. Autopsy confirmed the above diagnosis but showed no further malforma-

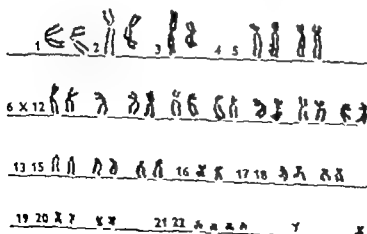


Fig 2 Karyotype of patient no 13 with a Y isochromosome or pericentric inversion of the Y chromosome

Table 1 (cont.)

Ref no	Age (yrs)	Sex chromatin (positive)	Chromosome counts					Total cells
			<45	45	45	47	>47	
187	10/12	39	4	4	38	1		47
194	1/12	40	3	2	40			45
195	20	40	1	5	50	1		57
196	21	33		1	43			44
200	43	28		2	38			40
201	23	19	2	3	36			41
205	41	2	1	3	44			48
206	1/12	2	1	2	43			46
207	2	24	1		39			40
210	35	38		3	33			36
212	25	29	1	1	33			35
215	1/12	2	2	2	47			51
219	1	27	1	1	38			40
224	30	24		1	39			40
233	9	38	1	3	37			41
263	1	18		4	47			51
276	0/12	25		4	35			39
283	0/12	36		5	35	1		41
287	6/12	1	1	2	51			54
288	5	21		2	33			35

and distribution of chromosome counts are seen in Table 1. However two out of the 82 patients showed minor chromosomal variations. In one of these two patients (no 263) a discrepancy in the size of the two chromosomes no 16 was found in all cells. No chromosome no 16 however was as big as a chromosome in group C or as small as a chromosome in group F. No chromosome studies were made among the members of the family of this patient. In the other patient (no 94) all cells examined showed an unusually long chromo-

somes in group C. The chromosome in question had a secondary constriction in the pericentromeric region of the long arm. The karyotype of the family was not examined.

The hypomodal cells showed no constant chromosomal pattern.

Definite chromosomal abnormalities were found in 18 out of the 100 patients. Twelve of these were phenotypic mongols, all with trisomy G without mosaicism (Table 2). Four of the 18 patients showed a clinical picture of Turner's syndrome and were all sex chromatin-

Table 2 Age, sex chromatin and distribution of chromosome counts for 12 patients with congenital heart disease and Down's syndrome

Ref no	Age (yrs)	Sex chromatin (positive)	Chromosome counts					Total cells
			<45	45	46	47	>47	
38	2	38	2	1	6	28		37
97	2/12	6			7	39		46
122	0/12	13	2	3	3	48		56
165	0/12	4	1	1	8	42		52
192	5/12	2		3	3	38		44
208	0/12	32	1	1	1	41		44
236	5/12	22	3	6	13	61		83
258	1/12	1	1		4	39		44
268	1/12	2	1		3	42		46
278	6/12	1			1	35		36
285	1/12	2	1		4	41	4	50
300	1	0			3	37		40

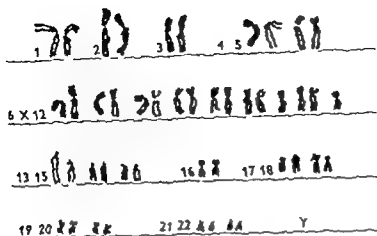


Fig. 5 Karyotype of the sister of patient no. 189 Translocation C/D

a broad flat nose low set ears deformities of the hands and feet and bilateral dislocation of the hip. Cardiac catheterization showed ventricular septal defect with considerable increase in pressure in the right ventricle. The patient was sex chromatin negative. A chromosome investigation showed a modal number of 46. In all the cells investigated one chromosome in group D was found to be considerably larger than the other chromosomes in the group (Fig. 4). The remainder of the chromosomes were normal. The mother's karyotype was normal but both father and sister had chromosome abnormalities as like the patient they had a large chromosome in group D but in addition

an unusually small chromosome presumably belonging to group C (Fig. 5). It may be assumed that in the father and sister this was a case of translocation C/D with maintenance of the normal amount of genetic material. In the propositus it may be considered that a translocation C/D is also involved but further more with partial trisomy for a chromosome in group C.

Table 5 shows the cardiovascular malformations found in relation to the karyotypes.

### DISCUSSION

Chromosome preparations are subject to many mutulating factors which in individual cells

Table 5 Cardiovascular malformations in relation to the karyotype

Cardiovascular malformation	No. of patients	Normal karyotype	Abnormal karyotype
ASD	15	15	2 trisomy C
PDA	14	14	1 trisomy C
T of F	12	11	2 trisomy C
A-V comm.	3	1	4 45,XO
C of A	5	1	5 trisomy C
ASD	20	4	1 trisomy C/D
Various	16	13	1 partial trisomy C
Type not verified	5	5	2 trisomy C
Total	100	82	1 no-Y

ASD = Atrial septal defect secundum type PDA = patent ductus arteriosus T of F = tetralogy of Fallot A-V comm. = patent aortic valvular intercommunicating C of A = coarctation of the aorta ASD = ventricular septal defect



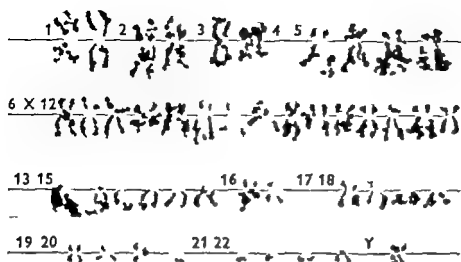


Fig 3 Autoradiography on preparations from the father to patient no 123

tions in the internal organs. A determination of sex chromatin in the cells of buccal smear showed that 6% of the cells contained a chromatin body. The chromosome analyses showed a modal number of 46 (Fig 2) and all cells contained 5 chromosomes in group F and only 4 chromosomes in group G+Y. The father, paternal grandfather and brother of the propositus all had the same karyotype, but the mother, the paternal grandmother and the 2 sisters of the father had normal karyotypes. Autoradiography was performed (Fig 3) on preparations from the father of the propositus and this showed that one of the chromosomes of the F group was considerably more late labelling than the others. This finding together

with the fact that the same chromosome abnormality was found in all male members of the family can best be explained by a Y chromosome abnormality. This could either be a Y isochromosome or a pericentric inversion of the Y chromosome.

The other patient with an unusual karyotype was a 2 month old boy (no 189) who was admitted for cardiac examination. His chromosome anomaly has already been described by Jensen & Melchior (8) who found the following: father, mother and an elder sister were phenotypically normal. The mother had had 2 abortions. There was no known congenital malformation in the family. The patient showed numerous malformations: cleft palate,

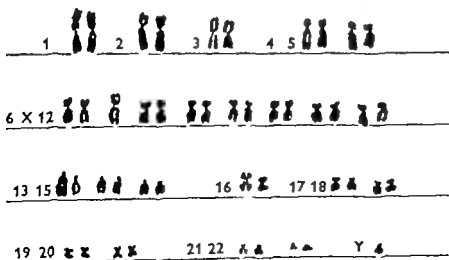


Fig 4 Karyotype of patient no 189 with translocation C/D and partial trisomy for a chromosome in group C

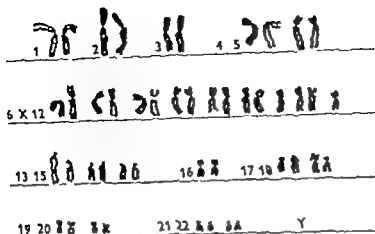


Fig 5 Karyotype of the sister of patient no 189 Translocation C/D

a broad flat nose low set ears deformities of the hands and feet and bilateral dislocation of the hip. Cardiac catheterization showed ventricular septal defect with considerable increase in pressure in the right ventricle. The patient was sex chromatin negative. A chromosome investigation showed a modal number of 46. In all the cells investigated one chromosome in group D was found to be considerably larger than the other chromosomes in the group (Fig 4). The remainder of the chromosomes were normal. The mother's karyotype was normal but both father and sister had chromosome abnormalities as like the patient they had a large chromosome in group D but in addition

an unusually small chromosome presumably belonging to group C (Fig 5). It may be assumed that in the father and sister this was a case of translocation C/D with maintenance of the normal amount of genetic material. In the proband it may be considered that a translocation C/D is also involved but further more with partial trisomy for a chromosome in group C.

Table 5 shows the cardiovascular malformations found in relation to the karyotypes.

### DISCUSSION

Chromosome preparations are subject to many mutilating factors which in individual cells

Table 5 Cardiovascular malformations in relation to the karyotype

Cardiovascular malformation	No of patients	Normal karyotype	Abnormal karyotype
ASD	15	13	2 trisomy G
PDA	14	14	
T of F	1	11	1 trisomy G
A-V com	3	1	2 trisomy G
C of A	5	1	4 45/XO
VSD	30	27	3 trisomy G
Various	16	13	1 translocation C/D
Type not verified	5	5	partial trisomy C
Total	100	87	2 trisomy G
			1 no-Y

ASD = Atrial septal defect secundum type PDA = patent ductus arteriosus T of F = tetralogy of Fallot A-V com = persistent ostium atrioventricular commune C of A = coarctation of the aorta VSD = ventricular septal defect

may influence the size of the chromosomes as well as their number or shape. The aim of the present study has not been a statistical evaluation of the incidence of chromosome breaks, heteromorphism, structural rearrangements, variations of satellite or aneuploidy. Is the occurrence of these abnormalities in individual cells may be caused by technical artefacts or may be the result of irradiation following angiocardiology or other roentgenological investigation. The present material therefore only includes those chromosomal abnormalities which occur in all cells with the modal number.

In the present material of 100 patients with congenital cardiovascular disease 16 patients had a classic syndrome—Down's syndrome or Turner's syndrome in which it is well known that congenital heart disease is a frequent occurrence. In Down's syndrome it is most often a case of persistent ostium atrioventriculare commune, atrial septal defect or ventricular septal defect. In the present material 9 of the 12 patients with Down's syndrome had one of these defects. The defect most often seen in Turner's syndrome is coarctation of the aorta which was present in all 4 patients with Turner's syndrome in this material.

The patient (no. 189) with translocation C/D and partial trisomy for a group C chromosome had in addition to heart disease numerous other congenital malformations. Only a few cases have been published of presumed partial trisomy of a chromosome in group C (4, 9, 10, 12) and a single case (5) with presumed pure trisomy of a chromosome in group C. In the cases with partial trisomy balanced reciprocal translocation involving a C group chromosome occurred in one or several family members who were phenotypically normal. The patients described in the above reports showed numerous different malformations and in some of them there was congenital heart disease in addition.

Secondary constriction of a chromosome is often seen particularly in group C and is a phenomenon without any special significance.

The secondary constriction can in all the cells in patient no. 94 cannot therefore be regarded as an aetiological factor in her heart disease (persistent ductus arteriosus).

It is improbable that there is any chromosomal explanation for her heart disease (ventricular septal defect) in the one patient (no. 263) with a discrepancy in the size of the 2 no. 16 chromosomes, as this phenomenon has often been described as an insignificant familial occurrence in otherwise normal persons (3).

In the patient with the Y chromosome abnormality other slight malformations were found in addition to the congenital heart disease—contractures of the fingers and a single crease on either little finger. However as the same karyotype was found in both father, brother and paternal grandfather without any accompanying malformations, this tells strongly against any relationship between the abnormal karyotype and the patient's heart disease. Presumed isochromosomy for the Y chromosome was described in 1966 (14) in father and son who were not reported to have shown other malformations. However both these patients were unusually tall—182 and 185 cm, respectively. Another case of presumed isochromosomy was described in 1966 (3) in a man who was 152 cm tall. The fact that the father and the paternal grandfather of the patient were of normal height perhaps tells in favour of the presence in this case of a pericentric inversion of a relatively large Y chromosome.

With the present cytogenetic technique as well as the inability to demonstrate minimal chromosomal abnormalities microscopically the possibility cannot be excluded of a causal connection between chromosomal abnormalities and congenital cardiovascular disease. In the present material however it has not been possible to reveal any chromosomal abnormality in patients with congenital heart disease where the latter was not associated with known syndromes or severe extra cardiac malformations.

## SUMMARY

Chromosome studies were made in 100 patients with congenital cardiovascular disease. Twelve patients had Down's syndrome with trisomy G. Four patients had Turner's syndrome with karyotype 45/XO. In one patient isochromosomy or pericentric inversion of the Y chromosome was found and was also found in the paternal grandfather, the father and the brother none of whom had malformations. One patient who also had severe extracardiac malformations had partial trisomy of a group C chromosome and translocation C/D. Translocation C/D was found in the father and sister who were phenotypically normal. In the remaining 82 patients normal chromosomal conditions were found although 2 patients had presumably insignificant chromosomal variations.

## ACKNOWLEDGEMENT

I wish to thank Dr Margareta Mikkelsen for performing the autoradiography and for her continued encouragement and advice. I am grateful to Miss Hanne Fodden for assistance with the microscopic preparations.

## REFERENCES

- 1 Andersson J, Mörner E, Elizabeth C & Emanuel R. Chromosome studies in 156 patients with congenital heart disease. *Brit Heart J* 27: 756 1965.
- 2 Bæk J, A. Sanderson B & Zetterqvist P. Association between congenital heart malformations and chromosomal variations. *Acta Paediatr Scand* 50: 1 1961.
- 3 Court Brown W M, Buckton J, E. Jacobs, Patrick A, Tough I, Hibel M, Lennberg E. V & Hoot J. D. Chromosome studies on

adults. Cambridge University Press, Cambridge 1966.

- 4 Edwards J H, Fraumero M, Davies J, Jacobs J & Young R. B. Structural heterozygosity in man: analysis of two families. *Ann Hum Genet* 26: 163 1962.
- 5 El Aidi O S, Powell H C & Biesecker J J. Possible translocation in chromosome group 6-12 in a mentally retarded patient. *Lancet* i 700 1963.
- 6 Ement Ingrid de Groot, J. Vermaut P & Coroneo P. Chromosomal abnormalities and congenital heart disease. *Circulation* 36: 886 1967.
- 7 Fip and A. A. A micro-method for chromosome analysis on peripheral blood cultures. *Lancet* ii 131 1962.
- 8 James M K & Melchor I C. Possible partial trisomy of a chromosome of group 6 X 12 and familial translocation heterozygosity in a child with congenital abnormalities. *Develop Med Child Neurol* 9: 315 1967.
- 9 Lindsten J, Fraumero M, Kluge H P & Zetterqvist P. Metaphase and mitotic studies of a familial reciprocal translocation between two autosomes of group 6-12. *Cytogenetics* 4: 43 1965.
- 10 Mikkelsen Margareta, Mortensen Elve, Skakkebaek N E & Yarnes, Marna. Familial reciprocal translocation between a group C (12?) chromosome and a late labelling G chromosome. *Acta Genet* 18: 241 1968.
- 11 Rohde R A. The chromosomes in heart disease. *Circulation* 34: 484 1966.
- 12 Rohde R A & Catz B. Vertical transmission of a new group C (6/9) chromosomal syndrome. *Lancet* ii 838 1964.
- 13 Szalai M, Makino S & Kaji T. Chromosomal aberrations in congenital cardiovascular disorders in man. *Proc Japan Acad* 39: 183 1963.
- 14 Stockert M. A probable Y isochromosome in father and son of tall stature. *Lancet* ii 391 1966.

Submitted April 4 1969

Accepted May 20 1969

Knagsholmvej 38

2610 Rødovre

Denmark

Key words: Congenital heart disease, chromosomal aberrations.

## GLUCOSE GALACTOSE MALABSORPTION

### *Studies on the Intermediate Carbohydrate Metabolism*

G W MEEUWISSE and B LINDQUIST

*From the Department of Paediatrics University of Lund Lund Sweden*

The clinical picture of glucose galactose malabsorption (GGM) has been described recently in detail (15). The condition manifests itself mainly by severe watery diarrhoea from birth. This is caused by a defective absorption of the monosaccharides glucose and galactose. Small amounts of glucose are however absorbed or may be transformed from other compounds in the intermediate metabolism. It was therefore considered of interest to investigate whether glucose when it does enter the blood stream is normally metabolized. Galactose metabolism was studied similarly.

The fructose absorption is normal in GGM as observed in intestinal intubation studies (13). Remarkably oral fructose loadings usually resulted in marked increases of the blood glucose level (15) in contrast to what is normally seen. It was therefore considered worthwhile to study this phenomenon more thoroughly.

A preliminary report of these studies has been given (12).

### MATERIAL

The material comprised of 3 patients and 2 normal children. Some clinical data including information on the diets are given in Tables 1 and 2. The diets

Abbreviations: CHO carbohydrate; FTT oral fructose tolerance test; GGM glucose galactose malabsorption; GalTT intravenous galactose tolerance test; GTT intravenous glucose tolerance test; iv intravenous;  $t_{1/2}$  half life of injected carbohydrate.

listed here had been fed for at least 4 days (considerably longer in most cases) prior to the different studies.

Two patients (cases 1 and 2) suffered from GGM. Their histories have been described previously (15) then designated as cases 4 and 6 respectively.

The third patient (case 3) suffered from cystic fibrosis of the pancreas. The diagnosis was based on a typical clinical picture including failure to thrive, steatorrhea, hypoproteinemia, a chymotrypsin activity in the stools (9) corresponding to 10-14  $\mu\text{g}$  per g faeces, a sodium content of the sweat of 99-116 mEq/l and a normal xylose absorption test.

Controls for the intravenous galactose tolerance tests (GalTT) were provided by two children in a good nutritional condition and without signs of digestive or metabolic diseases.

### METHODS

*Intravenous glucose and galactose tolerance tests* were performed as rapid infusions of 15 g sugar per  $\text{sq m}$  of body surface area at the second GTT in case 2 the amount of galactose infused was however 8.4 g per  $\text{sq m}$  (0.35 g per kg body weight). The infusions were given within 2-3 min into a cubital or an external jugular vein following an overnight fast. Due to lack of time the first GalTT in case 2 was however done 3 hours after a GTT. The half life ( $t_{1/2}$ ) of injected carbohydrates was read from a semi-logarithmic plot drawn to fit as well as possible the blood concentration values obtained 15 min and onwards after the time of injection.

*Oral fructose tolerance tests (FTT)* were performed after an overnight fast with doses of 2 g per kg body weight given as a 10% solution in water.

Capillary blood samples were taken at suitable intervals for the determination of blood sugar levels. Duplicate samples were taken for the determination of the fasting blood glucose level. Whole blood was deproteinized using  $\text{Ba}(\text{OH})_2$  and  $\text{ZnSO}_4$  (20) after

Table 1 Clinical data on patients and controls subjected to intravenous glucose tolerance and galactose tolerance tests and the resulting half life values of injected carbohydrate

Subject	Sex	Age (months)	Height (cm)	Weight (kg)	Diet data	Calories		1 v test $t_{1/2}$ in min	
						Daily intake	from well absorbed CHO	GTT	GalTT
Case 1 (GGM)	♀	19	80	10.2	8 g fructose per day	900	4	85	—
Case 2 (GGM)	♂	10	74	8.4	42 g fructose per day	900	20	17	—
		13	81	10.0	30 g fructose per day	1000	12	32	11
		70	120	19.9	10 g fructose per day	1500	3	—	8
Control 1	♂	14	79	11.6	Ordinary mixed diet	1100	50	—	9.5
Control 2	♂	19	84	17.0	Ordinary mixed diet	1100	50	—	6.5

In addition the day prior to this 1 v GTT the patient received a long lasting glucose infusion for measurement of the renal reabsorption of glucose producing blood sugar levels up to 900 mg/100 ml the total amount of glucose infused was 32 g.

Ingested mainly in the shape of sucrose

confrontation the supernatant was subjected to analysis.

Fructose was analysed using a glucose oxidase method (4) Galactose was determined in a similar way using galactose oxidase as described previously (11-15). Fructose was determined by a method specific for keto-sugars (16).

## RESULTS

Fig. 1 illustrates the GTTs performed in the two patients with GGM. Case 1 who prior to the test had been living on a CHO poor diet including only 8 g per day of fructose given

as 200 g of a formula containing 4 fructose showed a slow removal rate of injected glucose (Curve I  $t_{1/2}$  = 85 mm). After 1 hour the removal rate increased but it was still slow compared with the normal values (19-45 mm) for her age (14). Case 2 who when his second 1 v GTT was done was of about the same size as case 1 metabolized the injected sugar at a faster rate (Curve II  $t_{1/2}$  = 32 mm). His diet contained 30 g of fructose per day. Some months earlier he had had another GTT at that time he consumed more fructose 42 g

Table 2 Clinical data on patients subjected to oral fructose tolerance tests and resulting increments of the blood glucose concentration

Patient	Sex	Age (months)	Height (cm)	Weight (kg)	Diet and drugs	Calories		1st hour blood glucose increment mg/100 ml
						Daily intake	from CHO	
Case (GGM)		2	59	3.8	Soy and cereal formula supplying 16 g fructose per day	400	16	32
		5	68	5.9	Cereal formula supplying 80 g fructose per day	800	40	9
		10	74	8.4	Mixed diet, 4 g fructose per day	850	11	33
Case 1 (cystic fibrosis)	♂	4	58	4.0	Neutragen no enzymes	335	90	50
		5	60	4.5	Pelargon pancreatic enzymes	6.5	40	7

In case the values refer to well absorbed CHO (fructose). In case 3 the percentage of calories has been derived from the total CHO content of the diet.

Neutragen (Mead Johnson) contains 8.5 carbohydrates partly sucrose and partly starch. Pelargon (Nestlé) when prepared as a 17 solution in water contains 37 lactose, 20 sucrose, 20 corn sugar and 14 starch altogether 91 carbohydrates.

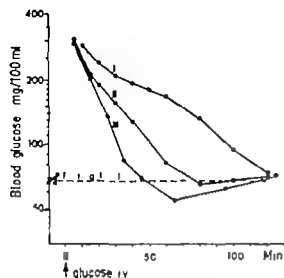


Fig 1 Intravenous glucose tolerance tests in two patients with glucose/galactose malabsorption. Curve I ( $t_1 = 85$  min) case 1 19 months 4 of caloric intake consisted of fructose. Curve II ( $t_1 = 32$  min) case 2 13 months 12 of caloric intake consisted of fructose. Curve III ( $t_1 = 17$  min) case 2 10 months 20 of caloric intake consisted of fructose furthermore the day prior to the test a large intravenous infusion of glucose was given. Note: An inverse correlation between  $t_1$  values and dietary intake of fructose was found.

per day and furthermore on the day before the test he received a low infusion of 32 g of glucose<sup>1</sup>. At that test he was found to have a fast removal rate of injected glucose (Curve III  $t_1 = 17$  min) just within the normal range of variation for his age (14).

The results of the iv GalTTs of case 2 and of the two controls are presented in Fig 2. In case 2 at 13 months of age the elimination of galactose from the blood ( $t_1 = 11$  min) proceeded more rapidly than the elimination of glucose but it was somewhat slower than in the controls who both had values within the normal range ( $t_1 < 10$  min) (19). All the subjects studied however presented a moderate rise in the blood glucose concentration as a response to the galactose injection indicating that in the patient as well as in the controls some galactose was converted to glucose.

This infusion which augmented the blood sugar concentration up to levels of 900 mg/100 ml was given for measurement of the renal tubular reabsorption of glucose.

At 6 years of age case 2 had a normal galactose removal rate  $t_1$  was 8 min and thus well within the normal standards of less than 12 min for children between 5 and 10 years of age (19).

The oral FTTs carried out in case 2 (GGM) and case 3 (cystic fibrosis) caused similar rises in the blood fructose concentration irrespective of the diets or treatments given (Figs 3 and 4 Table 2). The response of the blood glucose concentration was however variable in both cases. Flat curves were obtained when the patients were given sufficient calories and an ample supply of absorbable carbohydrates in the pre test diet. Higher responses were seen when the patients were deprived of carbohydrates. In case 3 at the first study (age 4 months) part of the dietary carbohydrates were badly utilized as starch could hardly be digested due to lack of pancreatic enzymes. The total supply of calories was also low. At this

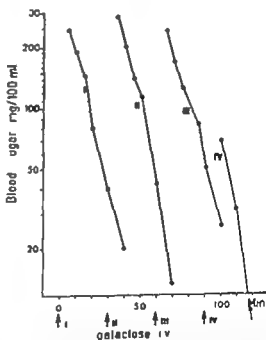


Fig 2 Intravenous galactose tolerance tests in two controls and one patient with glucose/galactose malabsorption. Solid lines blood glucose. Interrupted lines blood galactose. Curve I ( $t_1 = 9.5$  min) Control 1 14 months. Curve II ( $t_1 = 6.5$  min) Control 2 19 months. Curve III ( $t_1 = 11$  min) Glucose/galactose malabsorption case 2 10 months. Curve IV ( $t_1 = 8$  min) Same patient 6 years. At the latter test a smaller dose of galactose (in relation to body size) was injected than at the other tests.

of fructose (1 hour increments of 32 and 33 mg/100 ml respectively above the fasting level)

## DISCUSSION

The elimination curve of glucose obtained in case 1 at 19 months of age is very similar to that seen in healthy babies in the neonatal period. In newborn babies the glucose tolerance is low and furthermore in the second hour of an iv GTT there is usually an increase of the glucose removal rate just as observed in case 1 (2). The low glucose tolerance of newborn babies may be a consequence of the low blood glucose concentration of the foetus (5). Intravenous GTT's in severely malnourished infants also demonstrate a decreased rate of glucose removal (17). In adolescent boys (18) and in adults (3) too a dietary deprivation of carbohydrates has been shown to diminish the rate of removal of administered glucose from the blood.

Soon after the newborn period the glucose tolerance is higher than in adults (7, 14) but later on in infancy and childhood a gradual decrease in the glucose removal rate occurs (14). This latter development seems to be well correlated with the decrease in caloric intake in relation to body size of the growing individual of this intake carbohydrates supply a rather constant portion.

In the patients with GGM the results of the carbohydrate tolerance tests have demonstrated a relationship between the removal rate of glucose from the blood and the dietary supply of well absorbed carbohydrates. A low glucose tolerance is thus found when the diet contains very little fructose (case 1). Nevertheless a patient with GGM is capable of increasing the rate of removal of glucose from the blood when the intermediate metabolism has adapted to a larger supply of carbohydrates as demonstrated by the results of the iv GTT's in case 2.

It may be concluded that patients with GGM show the same pattern of reaction on variations

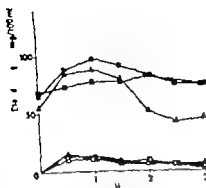


Fig. 3 Oral fructose tolerance tests in a patient with glucose galactose malabsorption (case 2). Symbols: 2 months 16% of dietary carbohydrates consisted of fructose,  $\Delta$  10 months 11% of dietary carbohydrates consisted of fructose,  $\square$  5 months 4% of dietary carbohydrates consisted of fructose.  $\circ$  on symbols blood fructose. Solid symbols blood glucose. Note: The supranormal rise of blood glucose was abolished by increased amounts of dietary fructose and it reappeared later on when the diet contained less fructose.

occasion the increase of the blood sugar concentration during the first hour was 50 mg/100 ml (Fig. 4). This finding was similar to the blood sugar rise observed in case 2 (Fig. 3) when his diet contained only small amounts

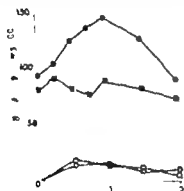


Fig. 4 Oral fructose tolerance tests in a patient with cystic fibrosis of the pancreas (case 3). Symbols: 4 months, low-caloric diet and no enzyme substitution,  $\bullet$  5 months, sufficient caloric intake and enzymes administered. Open symbols, blood fructose; solid symbols, blood glucose. Note: The supranormal rise of blood glucose was abolished by improved nutrition.



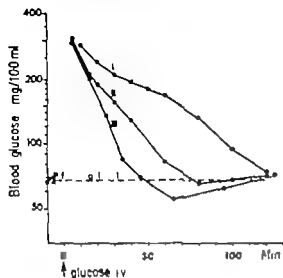


Fig 1 Intravenous glucose tolerance tests in two patients with glucose/galactose malabsorption. Curve I ( $t_1$  33 min) case 1 19 months 4% of caloric intake consisted of fructose. Curve II ( $t_1$  32 min) case 2 13 months 12% of caloric intake consisted of fructose. Curve III ( $t_1$  17 min) case 2 10 months 20% of caloric intake consisted of fructose. Furthermore the day prior to the test a large intravenous infusion of glucose was given. Note: An inverse correlation between  $t_1$  values and dietary intake of fructose was found.

per day and furthermore on the day before the test he received a slow infusion of 32 g of glucose<sup>1</sup>. At that time he was found to have a fast removal rate of injected glucose (Curve III  $t_1$  = 17 min) just within the normal range of variation for his age (14).

The results of the iv OGTT's of case 2 and of the two controls are presented in Fig 2. In case 2 at 13 months of age the elimination of galactose from the blood ( $t_1$  = 11 min) proceeded more rapidly than the elimination of glucose but it was somewhat slower than in the controls who both had values within the normal range ( $t_1$  < 10 min) (19). All the subjects studied however presented a moderate rise in the blood glucose concentration as a response to the galactose injection indicating that in the patient as well as in the controls some galactose was converted to glucose.

This infusion which augmented the blood sugar concentration up to levels of 900 mg/100 ml was given for measurement of the renal tubular reabsorption of glucose.

At 6 years of age case 2 had a normal galactose removal rate  $t_1$  was 8 min and thus well within the normal standards of less than 12 min for children between 5 and 10 years of age (19).

The oral FIT's carried out in case 2 (GGM) and case 3 (cystic fibrosis) caused similar rises in the blood fructose concentration irrespective of the diets or treatments given (Figs 3 and 4 Table 2). The response of the blood glucose concentration was, however variable in both cases. Flat curves were obtained when the patients were given sufficient calories and an ample supply of absorbable carbohydrates in the pre test diet. Higher responses were seen when the patients were deprived of carbohydrates. In case 3 at the first study (age 4 months) part of the dietary carbohydrates were badly utilized, as starch could hardly be digested due to lack of pancreatic enzymes. The total supply of calories was also low. At this

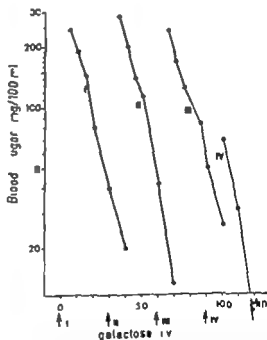


Fig 2 Intravenous galactose tolerance tests in two controls and one patient with glucose/galactose malabsorption. Solid lines blood galactose. Interrupted lines blood glucose. Curve I ( $t_1$  = 9.5 min) Control 1 14 months. Curve II ( $t_1$  = 6.5 min) Control 2 19 months. Curve III ( $t_1$  = 11 min) Glucose/galactose malabsorption case 2 13 months. Curve IV ( $t_1$  = 8 min) Same patient 10 years. At the latter test a smaller dose of galactose (in relation to body size) was injected than at the other tests.

malnourished signs of decreased glucose tolerance were seen with an oral fructose tolerance test. In him as well as in the patient with glucose galactose malabsorption the supranormal rise of blood glucose was abolished by dietary measures ensuring an ample supply of calories and carbohydrates.

The intravenous galactose tolerance tested in one of the patients was probably normal at 13 months of age and certainly normal at 6 years of age.

It is concluded that the observed abnormalities of the metabolism of glucose in patients with glucose galactose malabsorption are un-specific for this disease and merely secondary to carbohydrate deprivation.

### ACKNOWLEDGEMENT

The authors wish to thank Dr P. Hobbs, Department of Pediatrics, Central Hospital of Boston for performing the second galactose tolerance test of case 2.

### REFERENCES

1. Abraham J M, Lerner B, Oberholzer V H & Riesel A. Glucose galactose malabsorption. *Arch Dis Child* 47: 597 1967.
2. Bove M D, Melligan P B & Schwartz R. Intravenous glucose tolerance in the newborn infant: the effects of a double dose of glucose and insulin. *Pediatrics* 31: 590 1963.
3. Craig J W, Miller M, Mackenzie M S & Woodward H Jr. The influence of dietary carbohydrate deprivation on the metabolism of intravenously administered fructose and glucose in man. *J Clin Invest* 37: 515 1958.
4. Dahlqvist A. Determination of maltase and sucrase activities with a glucose-oxidase reagent. *Biochem J* 80: 547 1961.
5. Drury O S & Shelley H J. Physiological aspects of carbohydrate metabolism in the foetus and newborn. In F. Dickens, P. J. Randle and W. J. Whelan (eds). *Carbohydrate metabolism and its disorders*. Academic Press, London and New York 1964, vol. pp 101-103.
6. Donnet G W, Wren G Ng, Hodgman J E & Berggren W R. Galactose metabolism in the newborn infant. *Pediatrics* 39: 829 1967.
7. von Feyer U, Larsson Y & Persson B. Glucose tolerance in the neonatal period and during the first 6 months of life. *Arch Dis Child* 39: 393 1964.
8. Fromm I R, Frazer A, Labhart A, Stuber H W & Wolf H P. Die hereditäre Fruktosein-

- toleranz, eine bisher nicht bekannte kongenitale Stoffwechselstörung. *Schweiz. Med. Wochschr* 87: 1168 1957.
9. Haverback B J, Dyce B J, Gotting P J & Montgomery D W. Measurement of trypan and chymotrypsin in stool. A diagnostic test for pancreatic exocrine insufficiency. *Gastroenterology* 44: 588 1963.
  10. Hecrotsky A. A new method for the determination of urelin in plasma and urine. *Clin Chim Acta* 1: 470 1956.
  11. Kaba Laboratory Instructions. Address: KABI, Fack S 124 25 Stockholm, Sweden.
  12. Lindqvist B. Blockage of active transport of glucose. *Transactions XI Int Congr Paediatrics Group panel discussions* p III Tokyo 1965.
  13. Lindqvist B & Meunier G W. Intestinal transport of monosaccharides in generalized and selective malabsorption. *Acta Paediatr Scand* Suppl 146: 110 1963.
  14. Loebe H. Variations in glucose tolerance during infancy and childhood. *J Pediatr* 68: 237 1966.
  15. Meunier G W & Mitrin I. Glucose galactose malabsorption. A clinical study of 6 cases. *Acta Paediatr Scand* Suppl 188: 3 1969.
  16. Orsini M & Stopa T. Comportamento della glicemia fruttosemia e glucosemia dopo carico orale di fruttosio e saccarosio nel bambino normale. *Arch Ital Pediatr* 15: 122 1951.
  17. Orsini S V, Maccioni A S, Zucchi A C, Spada R G & Moockeborg F B. Disturbances of carbohydrate metabolism in infantile marasmus. *Am J Clin Nutr* 21: 1285 1968.
  18. Ieromon B, Sierky G & Thorell J. Effect of low carbohydrate diet on plasma glucose, free fatty acids, glycerol, ketones and insulin during glucose tolerance tests in adolescent boys. *Metabolism* 16: 714 1967.
  19. Ralander A. Intravenous galactose tolerance test. Normal series of children. *Scand J Clin Lab Invest* 2<sup>o</sup> 196 1968.
  20. Somogyi M. Determination of blood sugar. *J Biol Chem* 160: 69 1945.
  21. Tengstrom B. An intravenous galactose tolerance test with an enzymatic determination of galactose: a comparison with other diagnostic tests in hepatobiliary diseases. *Scand J Clin Lab Invest* 18 Suppl 92: 132 1966.
  22. Vink C L J. Liver function and age. *Clin Chim Acta* 4: 674 1959.

Submitted May 6 1969

Accepted May 17 1969

Barnhulten  
Lanzett  
Lund  
Sweden

**Key words:** Malabsorption, intravenous glucose tolerance test, intravenous galactose tolerance test, oral fructose tolerance test, influence of carbohydrate deprivation.

in the intake of absorbable carbohydrates as do normal children from the newborn period and onwards

In case 2 at 13 months of age the removal rate of intravenously administered galactose was slightly retarded as compared to the two controls. This is at variance with the finding of a normal elimination rate of galactose in an infant with GGM reported by Abrahim *et al* (1). Although not subjected to investigations aimed at the detection of subclinical damage there were no signs of damage of the liver parenchyma in our patient. The observation that orally given fructose which is well absorbed in patients with GGM produced values of the blood fructose concentration not exceeding 10–15 mg/100 ml does not support a suspicion of liver damage in that case; higher blood fructose levels would have been expected. Probably the half life of galactose of 11 min at 13 months of age is within the normal range of variation, despite the upper limit of 10 min found by Relander (19). He used a galactose dose of 0.35 g galactose per kg body weight which is about half the dose used here in children between 10 and 19 months of age. A comparison of the few publications on galactose tolerance tests in children (6, 19, 22) indicates a relationship between dosage and the resulting half life values. From these publications and preliminary studies in our department it seems that higher galactose doses produce larger half life values.

In normal individuals oral administration of fructose results in a very modest rise or sometimes even in a slight fall in the blood glucose concentration (8, 16). Hence although very helpful in the clinical diagnosis of GGM the observed rise in blood glucose concentration after the oral administration of fructose to patients with this disorder (15) cannot be regarded as a normal response to a FTT. Nor is this finding specific for GGM: the patient with cystic fibrosis (case 3) was found to react in the same way. In this patient however the rise of blood glucose was perhaps not only due to decreased absorption of dietary CHO

caused by disturbed digestion of starch but also to the low total supply of calories. In both cases a normalization of the FTT was seen when the diet contained more absorbable CHO. These observations are in agreement with experimental findings in adults subjected to CHO deprivation (3): the removal rate of intravenously injected fructose was not affected by the diet, but the resulting rise of blood glucose was found to be higher and more prolonged after several days of fasting or CHO deprivation than after a normal diet.

The studies presented here indicate that patients with GGM possess a normal ability to adapt their glucose metabolism to varying intakes of absorbable carbohydrates and that observed signs of a reduced rate in glucose metabolism are secondary to dietary factors. As the glucose metabolism normalizes on increasing amounts of fructose in the diet it is unlikely that in GGM the entrance of glucose into the liver and the peripheral cells is disturbed in a way similar to the entrance into the epithelium of the small intestine.

## SUMMARY

In 2 children with glucose galactose malabsorption the intermediate metabolism of hexoses was studied. The removal rate of injected glucose was slow when the diet of the children contained limited amounts of well absorbed carbohydrate (fructose). When sufficient fructose was given in the diet, the half life of injected glucose was normal. A fast removal rate was seen when large amounts of carbohydrates had been administered prior to the intravenous GTT. The results of oral fructose tolerance tests varied with the pre test diet in a similar manner.

The fructosemia was small and independent of the diet but the blood glucose concentration reacted in a way indicating decreased glucose tolerance if the diet contained little absorbable carbohydrate. This was evidently an unspecific effect of carbohydrate deprivation in a control case: an infant with cystic fibrosis initially

Table 1 Excretion of cystathionine and catecholamines in twenty infants and children with neuroblastoma

The values are expressed in  $\mu\text{g}$  per g creatinine n.d. = not determined

Case no.	Sex	Age (years)	Cystathionine in urine	3 Methoxy-4-hydroxy mandelic acid in urine	Methoxy catecholamines in urine	Metastasis
1	♂	1/12	140	230.0	13.5	Yes
2		3/12	250	121.7	9.1	Yes
3	♂	4/12	0	43.5	17.9	Yes
4	♂	6/12	230	171.0	51.2	Yes
5	♀	6/12	180	32.2	5.9	No
6	♂		140	199.0	n.d.	Yes
7	♂	1	0	25.6	2.0	No
8		1 3/12	0	37.2	5.7	Yes
9		1 4/12	100	44.3	4.7	No
10	♂	1 6/12	160	36.3	n.d.	Yes
11	♂	2	0	28.7	n.d.	No
12	♂	2 6/12	260	90.3	19.5	Yes
13	♂	6 2/12	40	71.3	3.1	Unknown
14	♂	3	40	29.7	7.2	Yes
15	♀	4	90	44.0	9.8	No
16	♀	4	0	26.5	8.0	Yes
17	♂	4 6/12	20	23.5	5.8	Yes
18	♂	6	220	769.2	80.1	Unknown
19	♀	6	0	75.3	19.8	Yes
20	♂	10 6/12	100	107.3	8.9	

logical amounts of 3 methoxy-4 hydroxymandelic acid and for methoxycatecholamines in urine. These values are also given in the table.

#### CYSTATHIONINE $\mu\text{g/g}$ Creatinine

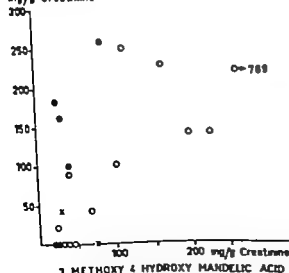


Fig. 1 Relation b/w between urinary excretion of cystathionine and 3-methoxy-4-hydroxymandelic acid in 20 children with neuroblastoma with metastasis (●) and without metastasis (○). — No record available about metastasis.

The figures in the table suggest a somewhat higher degree of cystathioninuria in children below the age of one year. There seems to exist a relationship between the grade of catecholamine production and the presence of metastasis. Because all 6 patients with a urinary excretion of 3-methoxy-4-hydroxymandelic acid greater than 100  $\mu\text{g}$  per g creatinine had metastasis (Fig. 1).

#### COMMENTS

The present investigation confirms the earlier reports of cystathioninuria in children with neuroblastoma. Since the publication of an increased number of patients with neuroblastoma the supposed relationship (2, 10) between cystathioninuria and the presence of metastasis has been found not to hold. It appears now that only 2 of 3 cases with known metastasis exhibited cystathioninuria. This figure may be somewhat less reliable because it is difficult to know for certain if metastasis are present or not. On the other hand, children with widespread metastases had no cystathioninuria. All 20 patients with neuroblastoma of the maternal

## CYSTATHIONINURIA IN CHILDREN WITH NEUROBLASTOMA WITH AND WITHOUT METASTASIS

WILFRIED v. STUDNITZ

From the Department of Clinical Chemistry and Pediatrics University of Lund  
Malmö General Hospital Malmö Sweden

Increased urinary excretion of the amino acid cystathionine has been observed. A certain syndrome has been recognized which is called primary cystathioninuria. It is an extremely rare inherited condition often combined with mental retardation and very probably caused by lack of activity of the cystathionine cleaving enzyme, cystathioninase (1).

Children with pyridoxine deficiency and diseases with gross hepatic failure have also been reported to excrete cystathionine in pathological amounts. In this group of secondary cystathioninuria lack or increased need of pyridoxine has been held responsible for the increased excretion of this amino acid. A third group of children with cystathioninuria has been described. This group consists of children with malignant tumours of the sympathetic nervous tissue (2, 7, 9, 10) or with hepatoblastoma (4, 13). Cystathioninuria in children with neuroblastoma was first described by Gjessing in 1963. This finding was confirmed in our laboratory (10) and by Shaw *et al.* (9). Gjessing's and our impression was that in these children there is a correlation between cystathioninuria and the presence of metastasis or the degree of malignancy of the tumour. The present investigation was undertaken to reevaluate the problem of metastasis and cystathioninuria based on a larger number of cases.

### MATERIAL

The material consisted of random collections of urine from 20 patients (12 boys and 8 girls) with

histologically verified neuroblastoma. The pattern of urinary excretion of methoxycatecholamines of this material has been published previously (11). The patients ranged in age from 1 month to 6 years. These urine samples originate from children of various hospitals and were collected during a 3 year period. The samples were acidified (pH 3) and had been stored at -20 up to 10 months before analysis.

### METHODS

The urinary amino acids were separated by high voltage paper electrophoresis combined with paper chromatography and cystathionine was measured as described before (12). Urinary methoxycatecholamines (metadrenaline and metnoradrenaline) were measured as described by Piarso (5). Urinary 3-methoxy-4-hydroxymandelic was determined as described by Piarso *et al.* (6).

### RESULTS

Fourteen of 20 children with neuroblastoma excreted increased amounts of cystathionine. The amounts excreted varied between 20 and 260 mg per g of creatinine (Table 1). In 4 of the 14 patients no metastasis could be detected at the time of collection of urine and in one patient the patients records contained no note about any metastasis. Six children with neuroblastoma exhibited no cystathioninuria. Of these children 3 had metastasis and in 2 no metastasis could be detected clinically. In one patient without cystathioninuria the patients records contained no note about any metastasis. All children exhibited increased catecholamine production, as reflected in the patho-

## CLINICAL AND BIOCHEMICAL GENETIC ASPECTS OF INTERMITTENT BRANCHED CHAIN KETOACIDURIA

### Report of Two Scandinavian Families

H W GOEDDE, U LANGENBECK, H BRACKERTZ, W KELLER,  
T ROKKONES, S HALVORSEN, RAGNHILD KJEL and BRITA MYRTON

From the Institute of Human Genetics University of Hamburg Hamburg Germany  
and the Paediatric Research Institute Rikshospitalet Oslo Norway

Since Pauling's description of sickle cell anaemia as a molecular disease in 1949 the study of haemoglobin anomalies has given an impressive picture of genetically determined variation and has made possible the recognition of the relationship between molecular defects and respective clinical disorders (13).

Investigations in the field of haemolytic anaemia have shown various types of glucose 6 phosphate dehydrogenases (G6PD) (17). Yoshida (18) succeeded in showing an amino acid substitution in a genetically determined G6PD variant. Recently many reports have been given on biochemical characteristics of enzyme variants e.g. pyruvate kinase (12).

Genetic defects of amino acid metabolism are studied to a minor extent mainly because

of the difficulties in obtaining sufficient material for enzymatic studies. Nevertheless recent reports have shown biochemical and clinical variation as a consequence of heterogeneity in diseases formerly believed to be entities for instance hyperovalurina, hyperphenylalanina and cyanurina (1, 14, 16).

In this paper we describe observations of Maple Syrup Urine Disease (MSUD) in two Scandinavian families who were affected with a variant of MSUD the intermittent type of MSUD (2, 4, 8, 11). These forms can be clearly distinguished biochemically and clinically from the so-called classical type first described by Menke in 1934 (10). The genetically determined enzyme defect in classic MSUD is shown in Fig. 1.

## CLINICAL PICTURE, LABORATORY DATA AND THERAPY

### Family Fa (Ped tree Fig. 2)

Clinical details from this family have been reported earlier (4, 8). The father of the proband (Fa II 3) and thus grandfather's two sisters (II 1 and 2) are Swedish, the other members are Norwegian.

L. Fa (II 1) a girl born June 1961 and the second child of unrelated and healthy parents. Since the age of 6 months she several times developed bilateral otitis media. During these attacks, often associated with high fever she never had any neurological symptoms. In Sept. 1966 (15 month-old) she was admitted to the

1. Institute of Human Genetics, University of Hamburg, Germany.

2. Present address: Department of Biophysics, Johns Hopkins University School of Medicine, Baltimore, Md. USA.

3. Present address: Department of Pediatrics, University of Oslo, Norway.

4. Present address: Research Institute, Rikshospitalet, Oslo, Norway.

The biochemical studies were supported in part by grants from Deutsche Forschungsgemeinschaft, Stiftung V. A. Wagner & Co. and Forschung Chem. when both the U. J. and D. B. are members of a fellow.

5. Present address: Deutsche Forschungsgemeinschaft.

presented here had increased amounts of catecholamines and/or their metabolites in their urine but only 14 of them had cystathioninuria. This means that the detection of cystathionine in urine from a patient with neuroblastoma need not indicate an early sign of neuroblastoma nor provide evidence of metastasis. Gjessing (3) found a high content of cystathionine in neuroblastoma tissue. Therefore the cystathioninuria in neuroblastoma patients has been related to this finding and the cystathioninuria has been understood as leakage from the tumour. A definite answer of the origin of cystathionine found in urine cannot be given until the tumour content of patients without cystathioninuria has been analysed for its cystathionine content. Therefore a certain degree of pyridoxine deficiency due to impaired liver function in the often cachectic patients and the presence of metastasis in the liver should be borne in mind as a possible cause for cystathioninuria. This should also be seen in the light that newborns and young children have a much greater need for pyridoxine than older children and adults. This may also be reflected in the present material in which the degree and the frequency of cystathioninuria was higher in the youngest children with neuroblastoma. Administration of large doses of pyridoxine could help to answer this question.

### SUMMARY

Cystathioninuria was demonstrated in 14 of 20 children (aged 1 month to 6 years) with neuroblastoma associated with increased catecholamine production. In 4 of these 14 patients no metastasis could be detected. Two of the 6 children without cystathioninuria had metastasis. Cystathioninuria appeared to occur more frequent in children with neuroblastoma in the age below 1 year. Cystathioninuria in neuroblastoma is not an early sign of the disease and is not a reliable sign of metastasis.

### ACKNOWLEDGEMENT

This work was supported by grants from the Swedish Cancer Society and the Faculty of Medicine, University of Lund.

### REFERENCES

1. Frimpter G. W. Cystathioninuria. In I. B. Stanbury I. H. Wyngaarden and D. S. Fredrickson (eds) *The metabolic basis of inherited disease*. McGraw Hill New York 1966 pp 409-419.
2. Gjessing L. R. Studies of functional tumours. II. Cystathioninuria. *Scand J Clin Lab Invest* 15: 474 1963.
3. — Studies of functional neural tumours. III. Cystathionine in the tumour tissue. *Scand J Clin Lab Invest* 15: 479 1963.
4. Gjessing L. R. & Maunitzen K. Cystathioninuria in hepatoblastoma. *Scand J Clin Lab Invest* 17: 513 1965.
5. Pisano J. J. A simple analysis for normetanephrine in urine. *Clin Chim Acta* 5: 406 1960.
6. Pisano J. J. Crout J. R. & Abraham D. Determination of 3-methoxy-4-hydroxymandelic acid in urine. *Clin Chim Acta* 7: 285 1962.
7. Ruide D. N. Cystathioninuria in the diagnosis of abdominal tumours. Abstract. *Clin Chem* 14: 786 1968.
8. Scriver C. R. & Hutchison J. H. The vitamin B deficiency syndrome in human infancy. Biochemical and clinical observations. *Pediatrics* 31: 240 1963.
9. Shaw K. N. F. Lieberman E. Koch R. & Donnell G. N. Cystathioninuria. *Amer J Dis Child* 113: 119 1967.
10. v. Studnitz. Sulfur containing amino acids in the urine of patients with tumours from sympathetic nervous tissue. *Scand J Clin Lab Invest* 17: Suppl. 86: 190 1965.
11. — Urinary excretion of methoxycatecholamines in neuroblastoma. *Scand J Clin Lab Invest* 21: 333 1969.
12. — Secondary cystathioninuria. *Acta Paediatr Scand* 59: 173 1969.
13. Voute P. A. & Widmark M. K. Cystathioninuria in hepatoblastoma. *Clin Chim Acta* 22: 373 1969.

Submitted April 29 1969

Accepted May 17 1969

Dept of Clinical Chemistry  
Malmö Allmänna Sjukhus  
214 01 Malmö  
Sweden

**Key words** Neuroblastoma catecholamines in urine cystathionine

## CLINICAL AND BIOCHEMICAL GENETIC ASPECTS OF INTERMITTENT BRANCHED CHAIN KETOACIDURIA

### *Report of Two Scandinavian Families*

H. W. GOEDDE, U. LANGENBECK, D. BRACKERTZ, W. KELLER,  
T. ROKKONES, S. HALVORSEN, RAGNHILD KIL and BRITA MERTON

From the Institute of Human Genetics, University of Hamburg, Hamburg, Germany  
and the Paediatric Research Institute, Rikshospitalet, Oslo, Norway

Since Pauling's description of sickle cell anaemia as a molecular disease in 1949 the study of haemoglobin anomalies has given an impressive picture of genetically determined variation and has made possible the recognition of the relationship between molecular defects and respective clinical disorders (13).

Investigations in the field of haemolytic anaemias have shown various types of glucose 6 phosphate dehydrogenases (G6PD) (17). Yoshida (18) succeeded in showing an amino acid substitution in a genetically determined G6PD variant. Recently many reports have been given on biochemical characteristics of enzyme variants e.g. pyruvate kinase (12).

Genetic defects of amino acid metabolism are studied to a minor extent mainly because

of the difficulties in obtaining sufficient material for enzymatic studies. Nevertheless recent reports have shown biochemical and clinical variation as a consequence of heterogeneity in diseases formerly believed to be entities for instance hyperoxaluria, hyperglycaemia and cytinuria (1, 14, 16).

In this paper we describe observations of Maple Syrup Urine Disease (MSUD) in two Scandinavian families who were affected with a variant of MSUD, the intermittent type of MSUD (2, 4, 8, 11). These forms can be clearly distinguished biochemically and clinically from the so called classical type first described by Menke in 1954 (10). The genetically determined enzyme defect in classic MSUD is shown in Fig. 1.

Institute of Human Genetics, University of Hamburg, Germany

Present address: Department of Biophysics, Johns Hopkins University School of Medicine, Baltimore, Md. U.S.A.

Ulrich Hospital, Oslo. Present address: Scitallanorhnet 1, Nydalen, Akershus, Norway

Paediatric Research Institute, Rikshospitalet, Oslo, Norway

The biochemical studies were supported in part by grants from Deutsche Forschungsgemeinschaft, Stiftung Volkswagenwerk, and Fonds der Chemischen Industrie. U. L. and D. B. are recipients of a fellowship from Deutsche Forschungsgemeinschaft.

## CLINICAL PICTURE, LABORATORY DATA AND THERAPY

### *Family Fa (Pedigree Fig. 2)*

Clinical details from this family have been reported earlier (4, 8). The father of the probands (father (II-3)) and his grandfather (two sons (II-1 and 2)) are Swedish; the other forebears are Norwegian.

*U. Fa (II-2)* a girl born June 1961 and the second child of unrelated and healthy parents. Since the age of 6 months she several times developed bilateral otitis media. During these attacks often associated with high fever she never had any neurologic symptoms. In Sep. 1962 (14 months old) she was admitted to the



presented here had increased amounts of catecholamines and/or their metabolites in their urine but only 14 of them had cystathioninuria. This means that the detection of cystathionine in urine from a patient with neuroblastoma need not indicate an early sign of neuroblastoma nor provide evidence of metastasis. Gjesung (3) found a high content of cystathionine in neuroblastoma tissue. Therefore the cystathioninuria in neuroblastoma patients has been related to this finding and the cystathioninuria has been understood as leakage from the tumour. A definite answer of the origin of cystathionine found in urine cannot be given until the tumour content of patients without cystathioninuria has been analysed for its cystathionine content. Therefore a certain degree of pyridoxine deficiency due to impaired liver function in the often cachectic patients and the presence of metastasis in the liver should be borne in mind as a possible cause for cystathioninuria. This should also be seen in the light that newborns and young children have a much greater need for pyridoxine than older children and adults. This may also be reflected in the present material in which the degree and the frequency of cystathioninuria was higher in the youngest children with neuroblastoma. Administration of large doses of pyridoxine could help to answer this question.

### SUMMARY

Cystathioninuria was demonstrated in 14 of 20 children (aged 1 month to 6 years) with neuroblastoma is related with increased catecholamine production. In 4 of these 14 patients no metastasis could be detected. Two of the 6 children without cystathioninuria had metastasis. Cystathioninuria appeared to occur more frequent in children with neuroblastoma in the age below 1 year. Cystathioninuria in neuroblastoma is not an early sign of the disease and is not a reliable sign of metastasis.

### ACKNOWLEDGEMENT

This work was supported by grants from the Swedish Cancer Society and the Faculty of Medicine University of Lund.

### REFERENCES

- 1 Frimpter G W. Cystathioninuria in I B Stanbury I B Wyngaarden and D S Fredrickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1966 pp 409-419.
- 2 Gjesung L R. Studies of functional tumours II Cystathioninuria. *Scand J Clin Lab Invest* 15 474 1963.
- 3 — Studies of functional neural tumours III Cystathionine in the tumour tissue. *Scand J Clin Lab Invest* 15 479 1963.
- 4 Gjesung L R & Mautzen A. Cystathioninuria in hepatoblastoma. *Scand J Clin Lab Invest* 17 513 1965.
- 5 Pirano J J. A simple analysis for normetanephrine in urine. *Clin Chim Acta* 5 406 1960.
- 6 Pirano J J, Crout J R & Abraham D. Determination of 3-methoxy-4-hydroxymandelic acid in urine. *Clin Chim Acta* 7 285 1962.
- 7 Ruze D N. Cystathioninuria in the diagnosis of abdominal tumours. Abstract. *Clin Chem* 14 766 1968.
- 8 Scriver C R & Hutchison J H. The vitamin B deficiency syndrome in human infancy. Biochemical and clinical observations. *Pediatrics* 31 240 1963.
- 9 Shaw K N F, Lieberman E, Koch R & Donnell G N. Cystathioninuria. *Amer J Dis Child* 113 119 1967.
- 10 v Studnitz. Sulfur containing imino acid in the urine of patients with tumours from sympathetic nervous tissue. *Scand J Clin Lab Invest* 17 Suppl 36 190 1965.
- 11 — Urinary excretion of methoxycatecholamines in neuroblastoma. *Scand J Clin Lab Invest* 21 333 1965.
- 12 — Secondary cystathioninuria. *Acta Paediatr Scand* 58 171 1969.
- 13 Voate P A & Wadman S A. Cystathioninuria in hepatoblastoma. *Clin Chim Acta* 22 373 1968.

Submitted April 23 1969

Accepted May 17 1969

Dept of Clinical Chemistry  
Malmö Allmän Sjukhus  
214 01 Malmö  
Sweden

Key words: Neuroblastoma; catecholamines in urine; cystathionine.

## CLINICAL AND BIOCHEMICAL GENETIC ASPECTS OF INTERMITTENT BRANCHED CHAIN KETOACIDURIA

*Report of Two Scandinavian Families*

H W GOEDDE U LANGENBECK ■ BRACKERTZ W KELLER  
T ROKKONES S HALVORSEN RAGNHILD NIL and BRITA MERTON

*From the Institute of Human Genetics University of Hamburg Hamburg Germany  
and the Paediatric Research Institute Rikshospitalet Oslo Norway*

Since Pauling's description of sickle cell anaemia as a molecular disease in 1949 the study of haemoglobin anomalies has given an impressive picture of genetically determined variation and has made possible the recognition of the relationship between molecular defects and respective clinical disorders (13)

Investigations in the field of haemolytic anaemia have shown various types of glucose 6 phosphate dehydrogenases (G6PD) (27) Yoshida (18) succeeded in showing an amino acid substitution in a genetically determined G6PD variant Recently many reports have been given on biochemical characteristics of enzyme variants e.g. pyruvate kinase (12)

Genetic defects in amino acid metabolism are studied to a minor extent mainly because

of the difficulties in obtaining sufficient material for enzymatic study Nevertheless recent reports have shown biochemical and clinical variation as a consequence of heterogeneity in diseases formerly believed to be entities for instance hyperonathuria hyperglycaemia and cystinuria (1 14 16)

In this paper we describe observations of Maple Syrup Urine Disease (MSUD) in two Scandinavian families who were affected with a variant of MSUD the intermittent type of MSUD (2 4 8 11) The forms can be clearly distinguished biochemically and clinically from the so called classical type first described by Menke in 1954 (10) The genetically determined enzyme defect in classic MSUD is shown in Fig 1

1 Institute of Human Genetics University of Hamburg Germany

■ Institute of Biophysics Johns Hopkins University School of Medicine Baltimore MD USA

Oslo Hospital Oslo Prentiss Street Sentralla bokstaben i lybyskalete et Almond Norway

Paediatric Research Institute Rik Hospital Oslo Norway

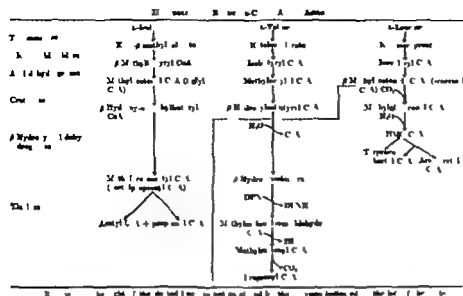
The biochemical studies are supported in part by grants from Deutsche Forschungsgemeinschaft Stiftung Volkswagenwerk and Fonds der Chemischen Industrie U L and B M are recipients of a fellowship from Deutsche Forschungsgemeinschaft

### CLINICAL PICTURE LABORATORY DATA AND THERAPY

*Family Fa (Pedigree Fig 2)*

Clinical details from this family have been reported earlier (4 8) The father of the probands father (II 3) and the grandfather's two sisters (II 1 and 2) are married the other members are non-consanguine

U Fa (II 2) a girl born June 1961 and the second child of unrelated and healthy parents Since the age of 6 months she several times developed bilateral otitis media During these attacks often associated with high fever she never had any neurologic symptoms In Sept 1962 (15 month-old) she was admitted to the



**Fig 1** Metabolism of the branched chain amino acids. The block in MSUD is localised in the second step of degradation.

hospital with a 3 day history of bilateral purulent otitis media. The first day she had fever (39 C), the second day she was febrile but then she got a series of brief tonic convulsions and became progressively lethargic. On admission she was febrile, semicomatose and had a strong curry odor. She had a metabolic acidosis with total CO<sub>2</sub> 7 mEq/l. Urinary chromatography revealed excessive excretion of leucine and valine. She remained unconscious for three days and had during this time several attacks of tonic and clonic convulsions with tonic spasms of the extremities.

The convulsions were treated with luminal and chloral. She gradually responded to intravenous infusion of electrolytes and glucose and was discharged well.

The past history has been noncontributory. She has had several acute infections without neurologic symptoms. At present she is 7 1/2 years old and of normal weight, height and intellectual development. The amino acid levels in serum and urine have been examined by chromatography on several occasions and have always been normal. The mother has been told to restrict the intake of milk and to withdraw milk during periods of fever.

**B Fa (II 1)** born March 1955 the 6-year-old brother of the preceding case. He was admitted to the hospital 8 months of age because of otitis media, fever, vomiting and diarrhea. Except the vomiting there were no other neurologic symptoms or signs. Apart from this one episode and a few periods in wintertime of acute bronchitis he was healthy and developed well physically and mentally. In school he was considered one of the brightest boys in the class. In Jan 1963 he was again admitted to the hospital under suspicion vomiting and lethargy. On the admission he was afebrile, semicomatose with high pitched cries and with frequent tonic spasms of the extremities. He had metabolic acidosis with total  $\text{CO}_2$  9 mEq/l. There was a strong curry like smell of the urine reminding of the odor of the sister when she was comatose 4 months previously.

Despite intravenous infusion of fluids and electrolytes he lapsed into coma. He responded poorly to anticonvulsive treatment. Because of respiratory failure he was intubated and put on artificial respiration and later a tracheostomy was performed. In addition there was a cardiovascular failure and he was kept on Aramine the last two days. He died 5 days after admission.

By chromatography of urine and serum there was found an excessive excretion of valine and leucine/isoleucine and a great elevation of the same amino acids in serum. Excessive amount of  $\alpha$  ketoglutaric acid (from leucine) and/or  $\alpha$  keto  $\beta$  methylvaleric acid (from isoleucine) were found in urine.

Autopsy revealed spongy degeneration of the deep layers of cortex and necrosis of the granule cell layer of the cerebellum. There was also a considerable nerve cell loss in the pontine nuclei and in substantia nigra.

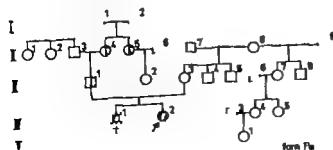


Fig. 2 Pedigree of the family Fa<sup>1</sup> first described in 1964 (4, 8)

Symbols used in Figs 2 and 3 not studied ☐  
 studied normal enzyme activity ☒ studied enzyme  
 activity about 50% of normal ☒ patients

*The other members of the family*

There has been no anamnestic clue of early death of other children in the family nor of neurologic attacks of any kind.

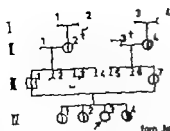


Fig. 3. Pedigree of the family Ja.

**Family Ja (Pedigree Fig. 3)**

**Case 1, Ja (I-2)** A girl born September 1944 as the third of four sisters of unrelated parents. The child was born at term after a normal pregnancy and has developed normally. She has been healthy except for the two episodes described in the following. At 1 year she had an episode of fever when she became oporotic. The mother observed a peculiar smell from the nappies.

At the age of two years she got an upper respiratory infection and had a temperature between 39 and 40°C for two days. The following day she was febrile but refused to eat and had periods of unconsciousness. She lay in oporotosis and was referred to the Paediatric Department, Rikshospitalet, Oslo with the diagnosis of meningitis. On admission she was cerebrally affected and without consciousness. The deep tendon reflexes were absent. She reacted to pain but not to other stimuli.

Temperature 36.4°C. The cerebrospinal fluid was normal for cells, protein and sugar. Blood leucocytes were 19 900/mm<sup>3</sup>, blood sugar 37 mg/100 ml, blood pH 7.27, pCO<sub>2</sub> 24 mm Hg and standard bicarbonate 14 mEq/l. The urine had strong carry like smell suggestive of maple syrup urine disease and gave a markedly positive reaction with 2,4-dinitrophenylhydrazine. Amino acid chromatograms showed an increased amount of leucine, isoleucine and valine both in urine and serum and this was confirmed by qualitative determination on a Technicon amino acid analyzer. EEG the day after admission showed marked generalized dysrhythmia, which as diffuse organic cerebral involvement. She was treated with ACTH and intravenous fluids. Attempts to give her a strict

diet low in the branched chain amino acids were not successful and she was therefore given a milk free diet. She rapidly improved from the second day onwards. EEGs taken on the fourth day of hospitalization showed still marked dysrhythmia, on the 11th day normalization and on the 16th day it was normal. Amino acid chromatograms of urine and serum showed normalization after a few days and were normal one week after admission. She is now 5 years old and has had no other episodes of the disease. She is without neurological and mental symptoms. Psychological examination has shown that her mental development is above average for age.

## BIOCHEMICAL GENETIC INVESTIGATIONS

**Methods**

The activities of branched-chain keto acid oxidases were estimated in the leucocytes of the peripheral blood. Substrates were 1-C labelled branched chain alpha keto acids: alpha ketoglutaric acid (α-KG), alpha keto isovaleric acid (KIVA) and alpha keto beta methyl valeric acid (MEVA). The CO<sub>2</sub> produced was measured. Enzyme activities are dealt with as micromoles ketoacid converted to CO<sub>2</sub> by 16 million leucocytes in 3 hours at 37°C (5). The number of lymphocytes in the whole blood cell suspensions was determined in order to judge the enzyme activities more precisely for reasons described previously (7). Activities obtained from normal homozygotes for the classic form of MSUD are summarized in Table 1 (6).

**RESULTS**

**Family Fa** was investigated in 1967 and 1968 and **Fam Ja** in 1968. The results of these investigations can be seen in Figs. 2 and 3. In each family one of the parents (in family Fa mother III-3 and in family Ja father III-1) and all tested relatives of this particular parent showed normal enzyme activities in our heterozygote test. In contrast the father III-1 of the family Fa and the mother III-7 of family

Table 1. Conversion of branched chain ketoacids with enzymes from leucocytes of normal homozygous and heterozygous persons.

Ketoacid	Converted substrate (nM/mole 16 · 10 <sup>6</sup> leucocytes)					
	Normal homozygotes			Heterozygotes		
	n	Mean	Standard deviation	n	Mean	Standard deviation
α-keto-glutaric acid	71	3.35	0.72	39	1.47	0.46
α-keto-isovaleric acid	32	4.04	0.78	79	1.79	0.61
α-keto-β-methylvaleric acid	7	3.09	0.78	27	1.44	0.34

Table 2 Conversion of branched chain ketoacids with enzymes from leukocytes of patients with intermittent maple syrup urine disease

		Conversion of substrate (nmol/s/ 1.6 × 10 <sup>7</sup> leukocytes)		
		KIC	KIVA	MEVA
Fa IV 2	August 1967	0.98	1.38	0.24
	August 1967	0.58	1.61	1.01
	April 1968	0.07	0.68	0.26
Ja IV 3	April 1968	0	0	0

Ja had enzyme activities in the range of 50 per cent of normal value. Similar values were observed in some relatives of these two patients. Results of activity measurements in the two patients are given in Table 2. Patient Fa IV 2 displayed minimal activity in peripheral blood cells. The test has been repeated 9 months later with the same result. The conversion rate of KIC was the lowest of the three substrates. With our method patient Ja IV 3 showed no enzyme activity at all.

### DISCUSSION

Biochemical genetic findings suggest two differences between the classical and intermittent type of MSUD.

1 In the classical form both parents have decreased enzyme activities (about half the amount of normals) whereas in the intermittent form only one parent had decreased activity of the branched chain ketoacid oxidases.

This phenotype is inherited as the heterozygous manifestation of an autosomal gene. The other parent and his relatives showed all normal activity values in the heterozygote test.

2 Patients with the intermittent form sometimes or always have residual activities of the respective oxidases in peripheral blood cells (the turnover of the leucine derivative KIC is lowered the most) whereas in classical MSUD the atypical homozygous patients have no activity at all.

For interpretation of 1 Only standard activity measurements have been performed and therefore nothing can be concluded about the

underlying biochemical alterations in enzyme protein.

It is not clear whether parents with diminished enzyme activity are heterozygous for the allele of classic MSUD. There are several ways to explain normal activity in either parent.

(a) Homozygosity for the normal allele but this seems to be highly improbable.

(b) Parents with normal activity may be heterozygous for an allele controlling the synthesis of an enzyme with only minor alteration in substrate affinity. Similar observations have been made in some families with pyruvate kinase deficiency where small alterations in substrate affinity have been observed (12) so-called dominant inheritance is not to be assumed.

(c) The possibility exists that parents with normal activity may have an allele producing a polypeptide chain which combines with a normal gene product to form a normally functioning enzyme whereas in doubly heterozygous persons the patients a combination may occur resulting in synthesis of an enzyme with grossly impaired function leading to the clinical symptoms of MSUD. In complementation studies on bacteria and moulds such enzymes with normal activity have been found to be unstable in heat denaturation experiments (15).

For interpretation of 2 Connelly and co-workers consider the classic MSUD to be a defect only of KIC and MEVA oxidation both of which they assume to be catalysed by one single enzyme (3).

We have found in heterozygotes for classic MSUD a 50 per cent diminution of the activities of all three oxidase complexes of the catabolism of valine, leucine and isoleucine (Table 1) (6).

The results and our findings of a different distribution of KIC and MEVA oxidase activities in peripheral blood (7) are not in accordance with the existence of a single KIC/MEVA oxidase as proposed by Connelly *et al*. Observations in another child with intermittent MSUD (11) seem to point to an isolated defect of KIC oxidation in intermittent MSUD.

Diminution of the KIVA and MEVA-oxidation in such cases may be caused by competitive inhibition. However as yet we have no information on the size of the intracellular pool of ketoacids in classic and intermittent MSUD.

The failure to show any activity in leukocytes of the patient in family Ja during a period free of symptoms may be owing to technical difficulties because of a low yield of  $^{14}\text{C}$ . Another explanation at this time is not possible.

Mechanisms leading to acidotic attacks are of special interest. La Du (9) has shown that according to the special kinetic properties of phenylalanine hydroxylase an intermittent course of hyperphenylalaninaemia is to be expected if the mutant enzyme has a diminished affinity to the pteridine cofactor.

Further studies on normal and mutant branched chain keto acid oxidase are needed to clarify the respective mechanism in intermittent MSUD.

### SUMMARY

Intermittent branched-chain ketoaciduria was detected in two Norwegian families. All three patients had normal intelligence. One died during an acute acidotic attack at the age of 8 years.

The biochemical and clinical data in the two families suggest a separate variant of branched chain ketoaciduria. Possible mechanisms of genetic heterogeneity in this disorder are discussed.

### REFERENCES

- 1 Ando T, Nishida W, L. Gershen T, Gong L, Heiner D C & Aray P F Metabolism of glucose in the metabolic form of hyperphenylalaninaemia. *J. Inher. Dis.* 54 1968
- 2 La Du M J Maple syrup urine disease. *Arch. Fam. Med.* 5 401 1968
- 3 Bowler J A & Connolly J L Branched chain keto-acid metabolism. II Evidence for the common identity of a ketoacyl-CoA synthetase and a keto- $\beta$ -ketoacyl-CoA synthetase. *J. Biol. Chem.* 243 35 1969
- 4 Durrer J, Hutter J & Røttkötter T Intermittent form of branched-chain ketoaciduria variant of maple syrup urine disease. *New Eng. J. Med.* 76 84 1967

- 5 Goedde H W, Richter F, Hufner M & Seel B Arbeitsvorschrift eines vereinfachten Heterozygotentestes für das Abnormerkrankung. *Klin. Wochschr.* 42 818 1964
- 6 Goedde H W & Keller W Metabolic pathways in maple syrup urine disease. In W. L. Nyhan (ed.) *Amino acid metabolism and genetic variation*. McGraw Hill Book Comp. New York, Toronto and London 1967 p 191
- 7 Goedde H W, Langenbeck U & Brachtel D Detection of heterozygotes in maple syrup urine disease. Role of lymphocyte count. *Hum. Heredit.* 6 189 1966
- 8 Kul R & Røttkötter T Late manifestation, variant of branched-chain ketoaciduria (maple syrup urine disease). *Acta Paediatr. Scand.* 53 346 1964
- 9 La Du M J Genetic variation in metabolic disorders. In C. L. Nyhan (ed.) *Amino acid metabolism and genetic variation*. McGraw Hill Book Comp. New York, Toronto and London 1967 p 121
- 10 Neulen J H, Hutter P L & Crapp M New syndrome. Progressive familial infantile cerebral dysfunction associated with unusual urinary substances. *Pediatrics* 34 463 1954
- 11 Hutter J & Brachtel H To be published
- 12 Pajula D E, Valtonen W N, Saeghian M A, Kuffer D R, Reed C F & McIntyre O R An inherited molecular lesion of erythrocyte pyruvate kinase. *J. Clin. Invest.* 47 1929 1968
- 13 Porter W F & Lehmann H Molecular pathology of human haemoglobin. *Nature* 219 902 1968
- 14 Rosenbery L L Genetic heterogeneity in cystinuria. In W. L. Nyhan (ed.) *Amino acid metabolism and genetic variation*. McGraw Hill Book Comp. New York, Toronto and London 1967 p 381
- 15 Sudduth O Interallelic complementation in vivo and in vitro. *Biochem. Biophys. Res. Commun.* 21 249 1965
- 16 Williams H F & Smith L H Jr Lysine acyluria. A new genetic variant of primary hyperoxaluria. *New Eng. J. Med.* 78 333 1968
- 17 Homocystinuria of thiamine 6-phosphate dehydrogenase in man. *WJIO Techn. Rep.* 5 1967 366
- 18 Yoshida A A single amino acid substitution separates a aspartic acid between normal (B+) and the common Negro variant (A+) of human glucose 6-phosphate dehydrogenase. *Proc. Natl. Acad. Sci.* 57 835 1957

Submitted July 10 1969

Accepted Aug 27 1969

(H W G.)

Institute of Human Genetics  
University of Hamburg  
Martinstr. 52  
2 Hamburg 20  
Germany

**Key words:** Inborn errors of amino acid metabolism; maple syrup urine disease; genetic variation; valine leucine; molecular; branched chain ketoacids.

Table 2 Conversion of branched chain ketoacids with enzymes from leukocytes of patients with intermittent maple syrup urine disease

		Conversion of substrate (nmol mol <sup>-1</sup> s <sup>-1</sup> ) 16 × 10 <sup>6</sup> leukocytes)		
		KIC	KIVA	MEVA
Fa IV 2	August 1967	0.98	1.38	0.24
	August 1967	0.58	1.61	1.01
	April 1968	0.07	0.68	0.26
Ja IV 3	April 1968	0	0	0

Ja had enzyme activities in the range of 50 per cent of normal value. Similar values were observed in some relatives of these two parents. Results of activity measurements in the two patients are given in Table 2. Patient Fa IV 2 displayed minimal activity in peripheral blood cells. The test has been repeated 9 months later with the same result. The conversion rate of KIC was the lowest of the three substrates. With our method patient Ja IV 3 showed no enzyme activity at all.

### DISCUSSION

Biochemical genetic findings suggest two differences between the classical and intermittent type of MSUD.

1 In the classical form both parents have decreased enzyme activities (about half the amount of normals) whereas in the intermittent form only one parent had decreased activity of the branched chain ketoacid oxidases.

This phenotype is inherited as the heterozygous manifestation of an autosomal gene. The other parent and his relatives showed all normal activity values in the heterozygote test.

2 Patients with the intermittent form sometimes or always have residual activities of the respective oxidases in peripheral blood cells (the turnover of the leucine derivative KIC is lowered the most) whereas in classical MSUD the atypical homozygous patients have no activity at all.

For interpretation of 1 Only standard activity measurements have been performed and therefore nothing can be concluded about the

underlying biochemical alterations in enzyme protein.

It is not clear whether parents with diminished enzyme activity are heterozygous for the allele of classic MSUD. There are several ways to explain normal activity in either parent.

(a) Homozygosity for the normal allele but this seems to be highly improbable.

(b) Parents with normal activity may be heterozygous for an allele controlling the synthesis of an enzyme with only minor alteration in substrate affinity. Similar observations have been made in some families with pyruvate kinase deficiency where small alterations in substrate affinity have been observed (12) so called dominant inheritance is not to be assumed.

(c) The possibility exists that parents with normal activity may have an allele producing a polypeptide chain which combines with a normal gene product to form a normally functioning enzyme whereas in doubly heterozygous persons the patients a combination may occur resulting in synthesis of an enzyme with grossly impaired function leading to the clinical symptoms of MSUD. In complementation studies on bacteria and moulds such enzymes with normal activity have been found to be unstable in heat denaturation experiments (15).

For interpretation of 2 Connelly and co-workers consider the classic MSUD to be a defect only of KIC and MEVA oxidation both of which they assume to be catalysed by one single enzyme (3).

We have found in heterozygotes for classic MSUD a 50 per cent diminution of the activities of all three oxidase complexes of the catabolism of valine, leucine and isoleucine (Table 1) (6).

The results and our finding of a different distribution of KIC and MEVA oxidase activities in peripheral blood (7) are not in accordance with the existence of a single KIC/MEVA oxidase as proposed by Connelly *et al*. Observations in another child with intermittent MSUD (11) seem to point to an isolated defect of KIC oxidation in intermittent MSUD.

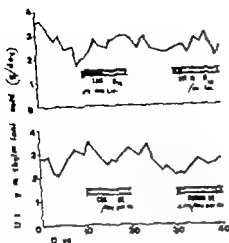


Fig. 1 Rate of urinary excretion of methylmalonic acid before and after treatment with 1 or 2.5 mg/day of vitamin B<sub>12</sub> and 2 or 4 mg/day per os of cobamide coenzyme

supplied by Recordati S.A.S. Milano (Italy) was also ineffective (see Fig. 1).

The metabolic block in the methylmalonic acidemia is in the two step conversion of methylmalonyl CoA to succinyl CoA (5). In the first step the D-methylmalonyl CoA is converted into its isomer by the enzyme methylmalonyl CoA racemase. In the second step the L-methylmalonyl CoA is transformed into succinyl CoA by methylmalonyl CoA mutase together with the cobamide coenzyme. There are no specific studies determining the precise site of the defect. However the decreased excretion of MMA after vitamin B<sub>12</sub> or cobamide coenzyme administration suggests that the block is in the L-methylmalonyl CoA → succinyl CoA reaction.

Nevertheless our observation points out the existence of a form of methylmalonic acidemia unresponsive to vitamin B<sub>12</sub>.

Therefore we are doubtful that methylmalonic acidemia can be simply considered a state of vitamin B<sub>12</sub> dependency and we think that there may be different genotypes of the disease.

This is in agreement with Auerbach's *et al* conclusions based on the enzymatic studies of

liver specimens of four patients with methylmalonic acidemia. They observed that none of the liver extracts of those four patients produced succinate from methylmalonate in the absence of vitamin B<sub>12</sub> and only one could normally form succinate in the presence of vitamin B<sub>12</sub>.

When a substantial number of patients will have been studied it will be possible to determine which are the clinical and biochemical differences between the vitamin B<sub>12</sub> responsive and unresponsive variants of methylmalonic acidemia.

## REFERENCES

- 1 Auerbach V H, Morrow G D, George A M & Barnes A L. Methylmalonic acidemia and glycemic variants: genotypes with enzymatic studies. Abstracts Federation of European Biochemical Societies, Sixth Meeting Madrid 7-11 4 1969 p 282.
- 2 Lindstedt B, Lindstedt L, Svanberg B & Zetterstrom R. The effect of cobamide coenzyme in methylmalonic acidemia. *Acta Paediatr Scand* 58 178 1969.
- 3 Oberholzer V G, Levin B, Burgess E A & Young W F. Methylmalonic acidemia. An inborn error of metabolism leading to chronic metabolic acidosis. *Arch Dis Child* 42 490 1967.
- 4 Paz, A A & Morris L. A modified procedure for the automatic analysis of amino acids. *Anal Biochem* 1 187 1960.
- 5 Rosenberg L, Lilljeqvist A Ch & Hsu Y E. Methylmalonic acidemia: metabolic block, localization and vitamin B<sub>12</sub> dependency. *Science* 167 805 1968.
- 6 Sotke O, Eldjarn L, Norum K R, Steen Johnsen I & Halvorsen S. Methylmalonic acidemia: A new inborn error of metabolism which may cause fetal acidosis in the neonatal period. *Scand J Clin Lab Invest* 20 313 1967.

Submitted June 19 1969

Accepted Aug 11 1969

(F Z)

Paediatric Clinic  
University of Padova  
Via Cristoforo 6  
35 100 Padova  
Italy

Key words: Methylmalonic acidemia, vitamin B<sub>12</sub> dependency, metabolic acidosis.



## SHORT COMMUNICATION

### METHYLMALONIC ACIDEMIA AND VITAMIN B<sub>12</sub> DEPENDENCY

F. ZACCHELLO and R. TENCONI

*From the Paediatric Clinic (Head: E. Sartori) University of Padova Padova Italy*

In a patient with methylmalonic acidemia a recently described inborn error of metabolism (3-6) Rosenberg *et al* (5) have reported a marked decrease of urinary excretion of methylmalonic acid (MMA) after daily parenteral administration of 1 mg of vitamin B<sub>12</sub>. In two other cases Lindblad *et al* (2) have found that MMA excretion during a tolerance test with valine was more than half diminished when 5 mg of cobamide coenzyme was given before the test.

Recently we have had the opportunity of giving large doses of vitamin B<sub>12</sub> and cobamide coenzyme to a child with methylmalonic acidemia. However we did not observe a decrease in the excretion of MMA.

II G, a boy was hospitalized the first time at birth in December 1965 because his three brothers had died between the third and fifth days of life of an unknown cause. On the fourth day of life the patient had a severe metabolic acidosis crisis with ketosis and recovered with parenterally infused fluids, sodium bicarbonate and glucose. During the next three years the child had 8 other severe episodes of ketoacidosis, often during an infection. In the intervals the child is clinically healthy and has only a mild compensated metabolic acidosis without ketonuria.

The infant has always been difficult to feed and refuses foods rich in proteins. His diet supplemented duly with 2 g of sodium bicarbonate has never had a protein content higher

than 2.5 g/kg. Physical and mental development is retarded. At 3 years of age the boy's weight was 11 kg, height 88 cm and his IQ 88. The liver and spleen are not enlarged. The lungs, heart, nervous system and muscle tone are normal. The MMA has been identified (for the first time in July 1968) by paper chromatography and determined according to Oberholzer *et al* (3). In the serum MMA ranged from 5 to 33 mg/100 ml and in the urine from 0.8 to 4.9 g/dry. The plasma glycine concentrations determined with an amino acid analyzer according to Picot & Morris (4) were 2.9 and 5.3 mg/100 ml during a ketoacidotic crisis and 9.6 and 13.5 mg/100 ml during a satisfactory clinical period. Plasma lactate levels vary from 14 to 64 mg/100 ml and the serum uric acid ranges from 6.9 to 17.1 mg/100 ml.

Mild anemia (Hb 8.4-10.6 g/100 ml, Hct 27-35) with normal or a little higher reticulocyte counts (1.2-2.7%) is usually present. During two acidosis crises we observed a severe anemia (Hb 5.4 and 5.1) and in one of them erythrocyte <sup>51</sup>Cr half-life was 12 days.

Sometimes we observed neutropenia but not thrombocytopenia. Megaloblastic changes were not present in the bone marrow.

No modification in MMA excretion was observed when the patient placed on a constant diet (2.5 g protein per kg) was given 1 or 2.5 mg/m<sup>2</sup> daily of vitamin B<sub>12</sub>. The administration of 2 or 4 mg per os daily of 5,6-dimethylbenzimidazole cobamide coenzyme (kindly

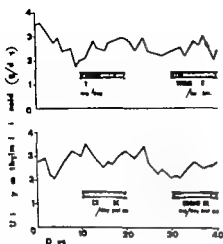


Fig 1 Rate of urinary excretion of methylmalonic acid before and after treatment with 1 or 2.5 mg/day 100 µg of vitamin B<sub>12</sub> and 2 or 4 mg/day per os of cobamide coenzyme

supplied by Recordati SAS Milano Italy) was also ineffective (see Fig 1)

The metabolic block in the methylmalonic acidemia is in the two step conversion of methylmalonyl CoA to succinyl CoA (5). In the first step the D-methylmalonyl CoA is converted into its isomer by the enzyme methylmalonyl CoA racemase in the second step the L-methylmalonyl CoA is transformed into succinyl CoA by methylmalonyl CoA mutase together with the cobamide coenzyme. There are no specific studies determining the precise site of the defect. However the decreased excretion of MMA after vitamin B<sub>12</sub> or cobamide coenzyme administration suggests that the block is in the L-methylmalonyl CoA → succinyl CoA reaction.

Nevertheless our observation points out the existence of a form of methylmalonic acidemia unresponsive to vitamin B<sub>12</sub>.

Therefore we are doubtful that methylmalonic acidemia can be simply considered a state of vitamin B<sub>12</sub> dependency and we think that there may be different genotypes of the disease.

This is in agreement with Auerbach *et al* conclusions based on the enzymatic studies of

liver specimens of four patients with methylmalonic acidemia. They observed that none of the liver extracts of those four patients produced succinate from methylmalonate in the absence of vitamin B<sub>12</sub> and only one could normally form succinate in the presence of vitamin B<sub>12</sub>.

When a substantial number of patients will have been studied it will be possible to determine which are the clinical and biochemical differences between the vitamin B<sub>12</sub> responsive and unresponsive variants of methylmalonic acidemia.

## REFERENCES

- 1 Auerbach V H, Morrow G D, George A M & Barncroft A L. Methylmalonic acidemia and glycemia: various genotypes with enzymatic studies. *Abstracts Federation of European Biochemical Societies, Sixth Meeting Madrid 7-11 4 1969* p 282.
- 2 Lindblad H, Lindstrand L, Svanberg B & Zetterstrom R. The effect of cobamide coenzyme in methylmalonic acidemia. *Acta Paediatr Scand* 58 178 1969.
- 3 Oberholzer V G, Levin B, Burgin E A & Young W F. Methylmalonic acidemia: An inborn error of metabolism leading to chronic metabolic acidosis. *Arch Dis Child* 42 492 1967.
- 4 Pez, A A & Morris L. A modified procedure for the automatic analysis of amino acids. *Anal Biochem* 1 187 1960.
- 5 Rosenberg L E, Lillqvist A Ch & Hsu Y E. Methylmalonic acidemia: metabolic block localization and vitamin B<sub>12</sub> dependency. *Science* 162 805 1968.
- 6 Seokke O, Eldjarn L, Norum K R, Steen Johansen J & Halvorsen S. Methylmalonic acidemia: A new inborn error of metabolism which may cause fatal acidosis in the neonatal period. *Scand J Clin Lab Invest* 20 313 1967.

Submitted June 18 1969

Accepted Aug 15 1969

(F Z.)

Pediatric Clinic  
University of Padova  
Via Giustiniani 6  
35 100 Padova  
Italy

Key words: Methylmalonic acidemia, vitamin B<sub>12</sub> dependency, metabolic acidosis.

## CASE REPORT

### ACUTE LEUKAEMIA WITH MEDIASTINAL INFILTRATE SIMULATING PERICARDITIS

G. A. M. DE VAAN and G. P. VOOYS

*From the Department of Paediatrics (Head E. D. A. M. Schreijen) and the Department of Pathological Anatomy (Head P. H. M. Schullings) Sint Radboudsche Universiteit  
The Netherlands*

Recently we had the opportunity to study a patient with a mediastinal leukemic infiltration simulating pericarditis. Because of the lack of information on this rather unusual clinical picture in the standard books of haematology we deemed it worthwhile to report this patient.

#### CASE HISTORY

P. T., a 12-year-old boy, was referred to us on May 10, 1966. A few days earlier he had been admitted to another hospital because of a bleeding tendency after a recent tonsillectomy. The consulting pediatrician diagnosed an acute leukaemia and a cardio-megaly and/or pericarditis. For further investigation he was sent to this hospital. In 1965 he had been treated successfully for a pulmonary tuberculosis.

On admission we found a sick boy in a good nutritional condition. The pulse was 100 per minute, rather well filled, blood pressure was 100/60 mm Hg. Respiratory movements were normal. The precordium was somewhat bulging. Cardiac dullness extended from the right sternal border to the left anterior axillary line. The cardiac sounds were very soft, pericardial rubbing or intracardiac murmurs were absent. The lungs were normal by auscultation and percussion.

The liver reached 5 cm, the spleen 4 cm below the costal border. Lymph nodes in the axilla, the groins and in the neck were small.

Laboratory investigations: ESR 13 mm, Hb 13.2 g/100 ml, thrombocytes 79 000/mm<sup>3</sup>. White cell count 250 000/mm<sup>3</sup> (73 blast cells). The urine was normal. The bone marrow smear (May-Grunwald-Giemsa stain) contained still normal cells of the erythroid and myeloid series. The majority were abnor-

mal; they had a diameter of 20 to 30  $\mu$  with dark nuclei, some of them containing 1 to 5 nucleoli. The cytoplasm was dark blue with vacuoles but contained no granules. About 30% of the blast cells were smaller with an average diameter of 10  $\mu$ . In the blood smear these smaller cells were more numerous. The diagnosis of acute leukaemia, probably of the myeloblastic type, was made.

X-ray investigations: on thorax X-ray an enlarged heart contour was seen. There was pleural fluid on the left side. Old healed calcified tuberculous lesions were present.

At fluoroscopy no cardiac pulsations were noted except at the lower right border. The upper mediastinum seemed to be broadened but no scalloping was seen either of the upper mediastinum or of the cardiac contours.

ECG: normal sinus rhythm, axi 60, PQ normal, QT 0.29-0.29 sec (normal). The ST segments were somewhat rounded. The T tops looked more normal than is found usually in a long-standing pericarditis.

Treatment and course: The diagnosis acute leukaemia was beyond all proof. We had no clear idea about the pericarditis-like picture. That this of tuberculous origin was seemed improbable, no signs of activity had been noted at any moment of follow-up.

We started treatment with vincristine, rubidomycin and prednisone and administered also antituberculous drugs. Two days after starting treatment the patient developed a fever of unknown origin. Blood cultures remained sterile. Completely unexpectedly he died on May 13.

#### Autopsy

At autopsy a large greyish white lobulated mass was observed within the anterior mediastinum. This mass extending from the left anterior cervical lymph nodes down to the diaphragm was 5 cm thick, where covering the heart and had a total weight of 400 grams.



Fig 1 Thorax X ray on admission

The tumor infiltrated the anterior wall of the pericardium. It reached up to the mesothelium without breaking through. The heart was laying free in the pericardial space and the cavity contained a normal amount of clear yellow fluid. The neoplasm covered the great vessels within the mediastinum.

At macroscopy the tumor cells were arranged in solid nodular fields divided by acellular fibrous septa. Two definite cell types could be recognized.

One type were large cells with a great round somewhat lobulated nucleus containing one or more nucleoli and a scarce pink cytoplasm. The chromatin was discrete and irregularly divided.

The other type were small cells with a small round hyperchromatic nucleus and a scarce pink cytoplasm. In these nuclei no nucleoli were found. Staining for acutely formed reticulum fibers were negative.

In an extensive study of many parts of the whole area no remains of preexistent thymic structures could be observed. The cytological picture of the

cells found in this mass suggests the same origin as the leukaemic cells that were noted in the bone marrow and elsewhere.

The pathological diagnosis was Extensive tumorous leukaemic infiltration of the anterior mediastinum. The regional lymph nodes were enlarged but nowhere grown together. In addition leukaemic infiltrates were found in the heart, liver, spleen, kidneys, adrenals and testes.

## DISCUSSION

Cardiac manifestations in acute leukemia do not receive great attention in the standard textbooks (3, 10, 11). The roentgenologic investigations in this patient tempted us to make the diagnosis of pericarditis. The electrocardiogram however did not favour this diagnosis.

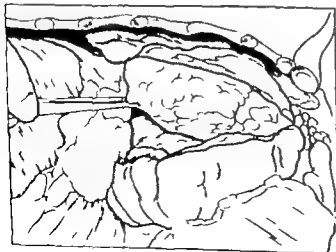


Fig 2 Pen drawing of the opened thorax at autopsy. The leukaemic infiltrate is divided into part of the anterior aspect of the pericardium is seen. No adhesions were present between the infiltrate and the smooth pericardium.

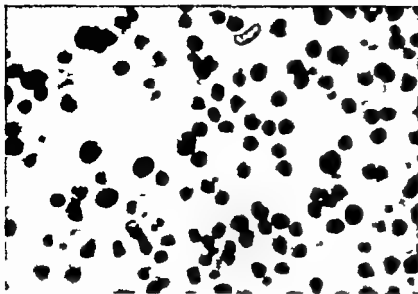


Fig. 3 Histology of the infiltrate (H & E Stain) Magnification 1600 two different cell types can be seen in this picture

Bierman *et al* (1) reported four patients with acute leukaemia who developed the clinical picture of pericarditis during the course of their disease. The electrocardiogram revealed tachycardia, low voltage, elevations of the ST segment and flat to inverted T waves. Haddy (4) described a 3 year old girl with acute leukaemia in which an enlarged heart was found with left pleural fluid. A presumptive diagnosis of congestive heart failure due to leukemic infiltration of the myocardium was made. The heart and the electrocardiogram became normal after cardiac irradiation. The usual treatment of cardiac failure with digitalis and diuretics tried before irradiation had had no effect. At autopsy 6 weeks after the clinical cure of the heart failure the epicardium was thickened but the pericardium and myocardium appeared normal.

Klatte *et al* (7) report a series of 341 patients with leukemia in which roentgenograms and clinical records could be studied. Hilar and mediastinal enlargement was often seen with clear demarcation of the lymph nodes so that there was scalloping of the margins. Only occasionally (5 cases) the mediastinal enlargement would be diffuse with smooth margins. One patient with superior vena caval obstruction was seen. Cardiac enlargement was noted often particularly in children with acute leukemia. In 35.5% autopsy was also performed.

Specifically they do not mention the presence of large mediastinal tumours. No special mention of it is made in the large clinical and pathological study of 123 cases of leukaemia of Hirschbaum & Preuss (5). Therefore it seemed very remarkable to us that the only report of similar cases in children dates back to 1932. Cooke described nine patients aged 3 to 13 years, all boys with a mediastinal tumour. Four had no leukaemic blood picture initially. The mass and the eventual initial dyspnoea disappeared after irradiation. In two patients autopsy was performed. These showed a big tumour mass extending from the thymic region over the pericardium. The tumours consisted of fields of mononuclear cells held together by connective tissue stroma. No thymic rests were found in these tumours. We believe that these patients have very much in common with our patient.

The mediastinal tumours are very sensitive to X ray (2). In our opinion it is not impossible that Haddy (4) actually reported a similar case that was treated successfully with irradiation. Kho Lien Keng (6) described a 6 year old boy with signs of tracheal compression caused by a superior mediastinal tumour, signs and symptoms disappeared after local irradiation. Some weeks later the blood picture became leukaemic.

Local irradiation seems to be of value antileu-

kaemic drug therapy may probably have the same good effect.

We cannot explain the sudden death of our patient Mathe *et al* (1968) mention the toxic effect of rubidomycin on the heart. But neither Mathe (8) or Massimo *et al* (9) report sudden deaths as a result of rubidomycin therapy. Rubidomycin was given by us to about 30 children without giving rise to cardiac complications.

We are tempted to believe that the tumour suddenly gave rise to serious circulatory and/or respiratory failure. A definite proof for this is however lacking.

While the diagnostic procedures during life always are limited, knowledge of the existence of such a clinical picture can lead to an early correct diagnosis.

## REFERENCES

1. Berman H R, Perlman E N & Ortega P. Pericarditis in patients with leukemia. *Amer Heart J* 43 413 1952.
2. Cooke J V. Mediastinal tumor in acute leukemia: a clinical and roentgenological study. *Amer J Dis Child* 44 1153 1952.
3. Dameshek W & Greut, F. *Leukemia*. Gracie & Strouton New York and London 1964 2nd ed.
4. Haddy T B. Cardiac irradiation in childhood leukemia. *Amer J Dis Child* 108 539 1964.

5. Kruhbaum J B & Prems P S. Leukemia: a clinical and pathological study of 123 fatal cases in a series of 14 400 necropsies. *Arch Int Med* 71 777 1943.
6. Nho Lien Hong. *Nieuwe aspecten van diagnostiek en therapie van leukemieën bij kinderen*. Thesis, Amsterdam 1952.
7. Klatte E, C. Yardley J, Smith E B, Robb R & Campbell J A. The pulmonary manifestations and complications of leukemia. *Amer J Roentgen* 89 598 1963.
8. Massimo L, Fomati Guglielmoni & Fortuna E. Daunomycin in the treatment of acute leukemia and neoplastic diseases in childhood. *Helv Paed Acta* 25 315 1968.
9. Mathe G, Hayet M, Schwarzenberg L, Annel J L, Schneider M, Cattani A, Schimberg J R & Jassim C. Haute fréquence et qualité des remissions de la leucémie aigüe lymphoblastique chez les enfants traités par l'association de delta 1-cortisone, l'interféron et le rubidomycine. *Arch Franç Ped* 25 181 1968.
10. Smith H. *Blood diseases of infancy and childhood*. C. V. Mosby Comp. St. Louis 1966 2nd ed.
11. Wintrobe M N. *Clinical Hematology*. Lea & Febiger Philadelphia 1967 6th ed.

Submitted May 14 1969

Accepted Aug 24 1969

(G d V.) Dept. of Paediatrics  
University of Nijmegen  
Geert Grooteplein Zuid 20  
Nijmegen  
The Netherlands

*Key words:* Acute leukemia, mediastinal infiltrate, pericarditis.

## CASE REPORT

### HEREDITARY ECTODERMAL DYSPLASIA

#### *Report of Two Cases*

GÖSTA SAMUELSON

*From the Department of Paediatrics County Hospital Lidköping and the Department of Paediatrics University Hospital Umeå Sweden*

Ectodermal dysplasia is usually characterized by the triad hypodontia hypotrichosis and hypo- or anhidrosis but other ectodermal manifestations are also seen (1 2 12) Up to the year 1947 80 cases had been published (16) and by 1967 about 300 (9)

From Sweden Ihre (11) described a child with anodontia in combination with other ectodermal stigmata In a clinical and genetic investigation Gribben (7) studied the frequency of hypodontia in a Swedish population In this series of about 2000 individuals no typical case of ectodermal dysplasia was found From Norway Rinvik & Syrrist (15) described one case of extreme hypodontia in connection with other ectodermal defects and Hasvold (8) two siblings with typical signs of ectodermal dysplasia From Denmark a number of case reports have been published (9 10 12)

Awawrad & Essawy (1) stressed the importance of being acquainted with the syndrome They particularly emphasized that infants with ectodermal dysplasia because they lack sweat gland can in high environmental temperature develop a feverish condition which can even result in death

In this paper two cases of ectodermal dysplasia are presented Both patients showed a very marked temperature instability

#### CASE REPORTS

*Case P B* a boy born September 22 1961 oldest of two children The parents and the younger child were healthy Wassermanns reaction of the mother was negative Pregnancy delivery and neonatal period were uncomplicated Birth weight was 3770 g and height 51 cm The boys maternal grandmother and great grandmother had a number of congenitally missing teeth and the grandmother had also noticed diminished perspiration Otherwise they and their relatives were healthy

The boys physical and psychomotor development was normal The parents noted early that he was very sensitive to heat exposure During warm and sunny days his temperature rose to 39°C without any signs of infection The mother noted that he could not perspire All the time he had severe nasal secretions and heavy crusting in the nose with a foul odour Furthermore at an age of he had only two erupted pear-shaped central primary incisors At this age he was admitted to the Department of Paediatrics at the County Hospital Lidköping

The physical examination revealed a normally developed boy without any infectious symptoms His weight and height were within the normal limits His ears were bowl-shaped and protruding The eyelashes and eyebrows were curly The scalp hair was fine blond and sparse His nasal bridge was flat and his lip protruding The finger and toenails were normal Beneath the eyes he had bilateral linear wrinkles His skin was dry and he had minor eczematoid lesions on the forearm

Haemoglobin concentration was 118 g/100 ml WBC was 9700 Differential leukocyte count showed a normal distribution MSR 11 mm PBI 70 µg/100 ml Cholesterol 171 mg/100 ml The serum protein electrophoresis was normal and so was the quantitative determination of immunoglobulins Cytogenetical

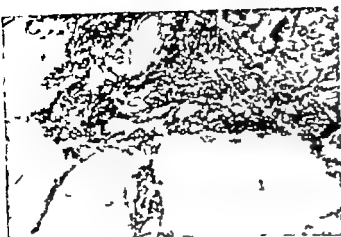


Fig 1 Section of skin (axillar region) from patient P.B. showing no sweat glands or sebaceous glands ( $\times 40$ )

examination of peripheral leucocyte cultures showed normal chromosome constitution. Urine examination for protein and glucose was negative.

A roentgenographic examination of the jaws showed underdeveloped alveolar processes. In the lower jaw there were no tooth buds and in the upper jaw only the primary central incisors and canines

(03+ 01+ +01 and +03) and the permanent central incisors (01+ +01) were detected. Only two primary teeth had erupted (01+ and +01) and both of them were peg shaped. The roentgenogram also revealed that the two permanent teeth (1+ and +1) were peg shaped.

Examination of the nose showed atrophic mucous membranes. Both nasal cavities were wide with heavy crusting. No special test of salivary or lacrimal gland function was made but the secretion seemed to be normal.

An experiment with careful heat provocation during continuous checking of the boy's temperature was performed. By means of electric pads placed on the bed the boy was exposed to increased heat for one hour and fifteen minutes. The rectal temperature increased during this time from  $37.1^{\circ}\text{C}$  to  $38.5^{\circ}\text{C}$  without any signs of perspiration except for very little on the front of the thighs. Another child of the same age simultaneously exposed to the same heat provocation showed strong perspiration. The rectal temperature of this child did not increase.

Biopsy of the skin was performed from the palm of the right hand and from the skin in axillar region for histopathologic examination. In serial sections from the biopsy material of the palm no sweat gland structures or sebaceous glands could be detected. In biopsy material from the axilla no sweat glands were seen except one underdeveloped and no fully developed sebaceous glands could be detected. No pathological changes were seen in the epidermis and the underlying corium contained the normal amount of hair follicles (P. A. Hofer) (Fig. 1).

After discharge from the hospital hyperpyrexia has been avoided through cooling measures.

A follow-up examination at the age of 8 years showed the same clinical picture as before (Fig. 2). The dental and skeletal age were delayed by about three years, but mental and physical development was normal. Roentgenograms showed as earlier hypodontia in the upper jaw and anodontia in the lower jaw (Figs. 3 and 4). A dental bridge had been



Fig. 2 The patient P.B. eight years old



## CASE REPORT

### HEREDITARY ECTODERMAL DYSPLASIA

#### *Report of Two Cases*

GÖSTA SAMUELSON

*From the Department of Paediatrics County Hospital Lidköping and the Department of Paediatrics University Hospital Umeå Sweden*

Ectodermal dysplasia is usually characterized by the triad hypodontia hypotrichosis and hypo or anhidrosis but other ectodermal manifestations are also seen (1 2 12) Up to the year 1947 80 cases had been published (16) and by 1967 about 300 (9)

From Sweden Ihre (11) described a child with anodontia in combination with other ectodermal stigmata In a clinical and genetic investigation Grijnen (7) studied the frequency of hypodontia in a Swedish population In this series of about 2000 individuals no typical case of ectodermal dysplasia was found From Norway Rinvik & Syrrist (15) described one case of extreme hypodontia in connection with other ectodermal defects and Hasvold (8) two siblings with typical signs of ectodermal dysplasia From Denmark a number of case reports have been published (9 10 12)

Awward & Essawy (1) stressed the importance of being acquainted with the syndrome They particularly emphasized that infants with ectodermal dysplasia because they lack sweat gland can in high environmental temperature develop a febrile condition which can even result in death

In this paper two cases of ectodermal dysplasia are presented Both patients showed a very marked temperature instability

#### CASE REPORTS

*Case P B* a boy born September 22 1961 oldest of two children The parents and the younger child were healthy Wassermann reaction of the mother was negative Pregnancy delivery and neonatal period were uncomplicated Birth weight was 3770 g and height 51 cm The boys maternal grandmother and great grandmother had a number of congenitally missing teeth and the grandmother had also noticed diminished perspiration Otherwise they and their relatives were healthy

The boys physical and psychomotor development was normal The parents noted early that he was very sensitive to heat exposure During warm and sunny days his temperature rose to 39°C without any signs of infection The mother noted that he could not perspire All the time he had severe nasal secretions and heavy crusting in the nose with a foul odour Furthermore at an age of 1 7 he had only two erupted pear-shaped central primary incisors At this age he was admitted to the Department of Paediatrics at the County Hospital Lidköping

The physical examination revealed a normally developed boy without any infectious symptoms His weight and height were within the normal limits His ears were bowl-shaped and protruding The eyelashes and eyebrows were curly The scalp hair was fine blond and sparse His nasal bridge was flat and his lip protruding The finger and toenails were normal Beneath the eyes he had bilateral linear wrinkles His skin was dry and he had minor erythematous lesions on the forearm

Hemoglobin concentration was 118 g/100 ml WBC was 9700 Differential leukocyte count showed a normal distribution MSR 11 mm PBI 70 µg/100 ml Cholesterol 171 mg/100 ml The serum protein electrophoresis was normal and so was the quantitative determination of immunoglobulins Cytogenetical

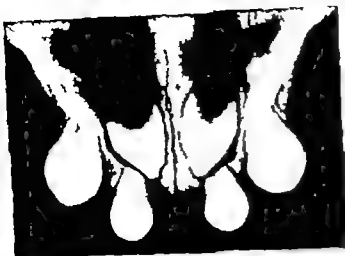


Fig 4 Roentgenogram of the upper jaw in case P B showing the pre-shaped teeth

only few cilia were found. The skull hair was fine blond and scanty (Fig 5). He had dry skin with slight eczematoid lesions on the cheeks and in the capillary tufts. Small spots of pigment defects were noticed suprapublically. The mucous membrane of the nose was atrophic. Routine haematological investigations and serum protein electrophoresis were normal. MSR was 11 mm. Cytogenetical examination of peripheral

lymphocyte cultures revealed a normal chromosome constitution. Roentgenographic examination of the upper and lower jaw showed no visible tooth buds.

A biopsy specimen was taken from the right axillary region. In serial sections no sweat glands could be detected. Hair follicles and sebaceous glands were seen but some of the sebaceous glands were obviously small in size (P. A. Hofer) (Fig 6).

During the hospital stay his temperature several times rose to 38-39°C. The boy's raised body temperature was easily controlled by sponging him with cooled water.

In October 1968 when he was 10 months old increased production of tears was noted by the mother. Tonometry revealed glaucoma on both sides and by gonioscopy bilateral malformation of the angle of the anterior chamber of the eye was diagnosed. Because of this a bilateral goniotomy was performed. Later a reoperation became necessary.

An odontological investigation including roentgenographic examination of the parents and seven siblings was performed but neither hypodontia nor abnormal shape of the teeth have been recorded.

## DISCUSSION

The two patients described presented all the main signs i.e. hypodontia, hypotrichosis and hypo- or anhidrosis as well as a great number of minor signs of ectodermal dysplasia. As minor signs many authors (1, 2, 16) have mentioned saddle-shaped nose, thickened and protruding lips, malformed and protruding ears, wrinkles around the eyes, pigment disturbances and atrophic rhinitis. These patients also often have eczematoid skin lesions similar to those described in the present patients. Of the minor signs the patients presented here lacked only



Fig 5 Facial appearance of the child G.Y. at eight months of age



Fig 3 Roentgenogram of the jaw in case P B Hypodontia in the upper jaw with peg shaped permanent teeth (1+ and +1) A dental bridge is inserted in the lower jaw no tooth buds are visible (a) Right side (b) Left side

made in the upper jaw when he was 7 years old. A plastic operation on the protruding ears had also been performed.

**Case G 1** a boy born December 25 1967. The parents were from the Lebanon. He had eight siblings. They were all healthy except one brother who was mentally retarded after an encephalitis. Wassermann's reaction of the mother was negative. The boy was born in Sweden one month prematurely. His birth weight was 2780 g and height 47 cm. There was no history of asphyxia.

He was admitted to another hospital at the age of 2 months because of unexplained fever attacks and difficulties in feeding.

The physical examination revealed protruding lips. The skin was dry and with slight eczematous manifestations. No infectious symptoms were detected. Routine haematological and urinary investigations

were normal. MSR 11 mm. Repeated bacterial blood cultures during attacks of high temperature were negative. Examination showed the cerebrospinal fluid to be normal.

During the 2 1/2 months hospital stay the boy had a short attack of gastroenteritis and a local skin infection. After treatment with antibiotics he became free of infection and it was then possible to observe him continuously during an infection free period. During this period he had repeated attacks of fever up to 40.5°C when the temperature of the room was increased. The child was discharged at the age of 5 months. Two months later he was admitted to the Department of Paediatrics at the County Hospital in Linköping because of fever attacks without any other signs of infection.

The physical examination revealed a small mouth protruding lips and a small nose. No eyebrows and

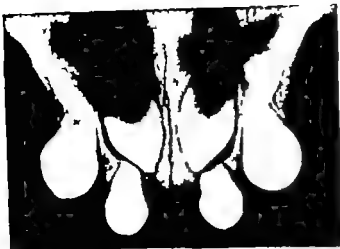


Fig 4 Roentgenogram of the upper jaw in case P B showing the peg-shaped teeth

only few cilia were found. The skull hair was fine blond and scanty (Fig 5). He had dry skin with slight eczematoid lesions on the cheeks and in the capillary tuft. Small spots of pigment defects were noticed suprapubically. The mucous membrane of the nose was atrophic. Routine hematological investigations and serum protein electrophoresis were normal. BSR was 11 mm. Cytogenetical examination of peripheral

leukocyte cultures revealed a normal chromosome constitution. Roentgenographic examination of the upper and lower jaw showed no visible tooth buds.

A biopsy specimen was taken from the right axillary region. In serial sections no sweat glands could be detected. Hair follicles and sebaceous glands were seen but some of the sebaceous glands were obviously small in size (P. A. Hofer) (Fig 6).

During the hospital stay his temperature several times rose to 38-39°C. The boy's raised body temperature was easily controlled by sponging him with cooled water.

In October 1968 when he was 10 months old increased production of tears was noted by the mother. Tonometry revealed glaucoma on both sides and by gonioscopy bilateral malformation of the angle of the anterior chamber of the eye was diagnosed. Because of this a bilateral goniotomy was performed. Later a reoperation became necessary.

An odontological investigation including roentgenographic examination of the parents and seven siblings was performed but neither hypodontia nor abnormal shape of the teeth have been recorded.

## DISCUSSION

The two patients described presented all the main signs i.e. hypodontia, hypotrichosis and hypo- or anhidrosis as well as a great number of minor signs of ectodermal dysplasia. As minor signs many authors (1, 12, 16) have mentioned saddle shaped nose, thickened and protruding lips, malformed and protruding ears, wrinkles around the eyes, pigment disturbances and atrophic rhinitis. These patients also often have eczematoid skin lesions similar to those described in the present patients. Of the minor signs the patients presented here lacked only



Fig 5 Facial appearance of the child III Y at eight months of age

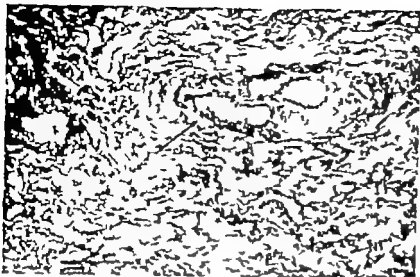


Fig 6 Section of skin (auricular region) from patient G Y showing no sweat gland structures but small sebaceous glands (→) ( $\times 100$ )

prominent frontal bossing and in case G Y the nose was small rather than saddle shaped. The nails as a rule are normally developed (1) and most often the patients are of normal intelligence (12).

Most striking was the instability of temperature regulation. Because they could not perspire both children developed high temperatures when the environmental temperature was increased. In the skin biopsies performed no fully developed sweat gland structures could be detected microscopically. A total absence of sweat glands or the presence of atrophic nonfunctioning glands is characteristic of ectodermal dysplasia with anhidrosis. In case P B it would probably be possible to detect sweat gland structures in the thigh region where perspiration was observed during the heat provocation test. In case G Y the very accentuated temperature instability speaks in favour of a complete anhidrosis. The lack of ability to perspire can be very dangerous (9). Jespersen (12) even described a child who died from hyperpyrexia during his first year.

Other gland structures can be involved; for example the lacrimal and salivary glands. The two cases described had no signs of impaired function of these organs. The sebaceous glands can also be lacking, but this is not a constant finding.

Case G Y had a malformation of the angle of the anterior chamber of the eye on both

sides resulting in bilateral congenital glaucoma. Malformations of eye structures of both ectodermal and mesodermal origin, i.e. the lens, the iris, the cornea, the angle of the anterior chamber of the eye are described in association with ectodermal dysplasia (4). However, the malformation of the angle of the anterior chamber of the eye is rare and seems to be described in only a few previous cases of ectodermal dysplasia (4, 6).

The peg shaped teeth seen in case P B are typical of the syndrome. The dentition could be delayed or absent as in case G Y. Because hypodontia can occur in other diseases, these conditions must be considered in the differential diagnosis. In incontinentia pigmenti (14) the shape of the teeth could resemble the peg shaped teeth of ectodermal dysplasia. The skin changes, however, are of another type and the patients have no inability to sweat. Ellis van Creveld's syndrome is a variant of anhidrotic ectodermal dysplasia. Hypodontia may be present, but this syndrome is associated with polydactyly, chondrodysplasia and congenital heart disease (13). Hypodontia is also found in patients with hidrotic ectodermal dysplasia described by Book (3), but these patients have hyperhidrosis and early whitening of the hair.

The genetic transmission has in some investigations been described as a sex linked recessive trait (5, 12). Other studies (8, 9) have shown a dominant transmission, often with m

complete penetrans and variable gene expressivity De Silva stated (5) that ectodermal dysplasia is extremely rare among girls. In the patients described here it has not been possible to prove fully developed ectodermal dysplasia among the parents or the siblings but in case P B both the maternal grandmother and the great grandmother had hypodontia and the grandmother also decreased ability to sweat. The mother had peg-shaped upper lateral incisors which can be a manifestation variation of hypodontia. However the frequency of peg-shaped upper laterals in a Swedish population is as high as two per cent (7). In the family of P B inheritance of the trait may be sex-linked recessive. Thus the boy's mother may be a heterozygote conductor of the trait. The mother and other female conductors have some ligature but not a fully developed syndrome. Another possibility is an autosomal dominant trait with variable expressivity.

The treatment of infants with ectodermal dysplasia is symptomatic. It is important to diagnose the condition as early as possible so that the parents can obtain information on how to prevent overheating. Cooling measures during high environment temperature and fever attacks are advisable and can be life saving. As in all cases of hypodontia it is necessary that dental development is checked by a pedodontist. Fixed and removable prostheses should be constructed for both functional and cosmetic purposes. Regular contact with an otolaryngologist may be necessary in treating the atrophic rhinitis.

### SUMMARY

Two cases of ectodermal dysplasia diagnosed at the ages of 2½ years and 7 months are described. The main signs were hypodontia, hypotrichosis and hypo- or anhidrosis. In both cases the temperature instability was a pronounced clinical problem. In one of the two cases bilateral glaucoma was diagnosed at the age of 10 months.

### REFERENCES

- 1 Arwaid S & Enayati H E. Hereditary anhidrotic ectodermal dysplasia. *Arch Pediatr* 77: 496 1960.
- 2 Blumner R I. Hereditary ectodermal dysplasia. Comments on current literature. *J Pediatr* 73: 444 1968.
- 3 Book J A. Clinical and genetical studies of hypodontia. *Am J Hum Genet* 2: 240 1950.
- 4 Chosack A & Rosenzweig L. Oligodontia associated with defects of the eyes. *Oral Surg* 21: 334 1966.
- 5 De Silva P C C. Hereditary ectodermal dysplasia of anhidrotic type. *Quart J Med* 8: 97 1919.
- 6 Gowler H & Bernhard H. Ein Beitrag zur Ektodermalen Dysplasie aus ophthalmologischer Sicht. *Acta Med Augmentin* 136: 57 1960.
- 7 Grahnén H. Hypodontia in the permanent dentition. *Odont Rev* vol 7. Suppl 3 1936.
- 8 Harvold P. Ectodermal dysplasia or anodontia. *Nordiskt Tandskiftet* Tid 65: 141 1955.
- 9 Happle E. Hereditary ectodermal dysplasia of anhidrotic type with increased protein bound serum thyroxine. *Acta Paediatr Scand* 56: 687 1967.
- 10 Holst G. Dental treatment of patients with ectodermal dysplasia. A report of five cases. *Tandlägebladet* 67: 60 1963.
- 11 Ilmarinen B. Case of anodontia. *Acta Med Scand* 79: 1 1932.
- 12 Jespersen H G. Hereditary ectodermal dysplasia of anhidrotic type. *Acta Paediatr Scand* 51: 712 1962.
- 13 Loden H & Sjögren I. Chondroectodermal dysplasia (Ellis van Creveld's syndrome). *Acta Paediatr Scand* 53: 583 1964.
- 14 Oldfelt V. Incontinentia pigmenti. *J Pediatr* 34: 446 1959.
- 15 Rönkä R & Syrjäst A. Medfödd tannmängd förbättrat med förändringar i baktärysmek. *Nordiskt Tandskiftet* Tid 49: 237 1939.
- 16 Sjöström B & Sjöström A. Hereditary anhidrotic ectodermal dysplasia. *Arch Dermatol Syph* 60: 1170 1949.

Submitted June 6 1969

Accepted July 4 1969

(G S) Dept Paediatrics  
Linnarctet  
Umeå  
Sweden

Key words: Ectodermal dysplasia, hypodontia, anhidrosis, congenital glaucoma.

# PROCEEDINGS OF PAEDIATRIC SOCIETIES

## SCANDINAVIAN SOCIETY OF PAEDIATRIC PATHOLOGY

Meeting in Copenhagen Denmark June 12-14 1969

Aagot Christie Løken (Oslo Norway) *Poliodystrophia cerebri progressiva infantilis*

*Poliodystrophia cerebri progressiva infantilis* is one name for a cerebral degeneration characterized by a diffuse loss of neurons throughout the cortex with a corresponding reactive astrogliosis. Involved to a lesser degree are the cerebellar cortex, the thalamic nuclei, the striatum and other nuclei. A progressive atrophy involving the white matter as well follows in prolonged cases.

The condition is seen in children who after a normal pregnancy and an uncomplicated delivery and postnatal period behave normally until a change in psychomotor development follows. There is a regression often complicated with jerks, rigidity and involuntary movements. Convulsions often complicate the picture until the child dies decerebrated in opisthotonus. The condition lasts from weeks to months or years. Two groups can be distinguished: one infantile with symptoms starting before 1 year, lasting up to 3 years of age; the second or juvenile with an onset between 3 and 8 years, lasting up to adult age.

**Case 1** A boy with a long history of vomiting and obstruction from 2-3 weeks of age gradually showed psychomotor retardation. Convulsions were at no time observed. He was hospitalized for malabsorption when he was 4 months old. He was treated in a home for mentally retarded children and was readmitted to hospital under the diagnosis of gastroenteritis shortly before he died 2 years and 7 months old. At autopsy there were no macro-

scopic changes in the viscera. The brain weighed 1160 g, appeared edematous with a moderately enlarged ventricular system and a thin corpus callosum. Extensive neuronal loss was found all through the cortex with astroglial proliferation and small sudanophilic microglia. Similar changes occurred in the striatum and globus pallidus. In the cerebellum there was loss of Purkinje cells and fibrillary gliosis. Some demyelination was seen in the temporal lobe.

**Case 2** A 17-month old boy with an unremarkable history until 16 months old when he had a cold with fever. The same day he had tonic and clonic convulsions and was admitted to hospital where he became comatose. EEG showed a diffuse mostly right-sided dysrhythmia. He became decerebrated and died after 4 weeks. The histopathological changes were very similar to those reported in the first case except that the temporal lobes and hippocampus were spared.

The etiology was discussed. Anoxia is usually suspected although the first case gave no clinical indication for this. Familial occurrence has been reported. Of other possibilities were mentioned defective fetal development, metabolic disorders, infections, virus, lack of vitamin B<sub>1</sub>.

It was concluded that although anoxia could probably produce a similar picture, there are several reasons for suspecting some other primary factor. It is also possible that various metabolic or other pathogenetic mechanisms may result in similar morphological pictures.

Arne Bruu (Lund, Sweden) *Aspects of cerebral development and maldevelopment of particular relevance to pediatric pathology*

Normal cerebral developmental features of premature and fullterm infants may simulate a pathological process. These features are:

1 Remnants of the periventricular matrix normally seen at fullterm consist of lymphocyte like neuroblasts and glial cells located lateral to the occipital and frontal ventricular horns and in the strio-thalamic groove. Last to vanish toward the 4th postnatal month are perivascular collars of lymphocyte like cells in the frontal lobes. The cerebellar nodulus often contains a mixture of nerve cells and undifferentiated cells.

2 Around birth the subpial granular layer (SGL) of the cerebral molecular layer remains in the temporal lobes particularly the uncus sometimes in the frontal poles and the prepyriform areas where the cells may appear to invade the meninges. It consists of glial and neuroblast like cells forming a layer subpially.

3 Together with cellular and vascular meninges these features may simulate inflammatory changes, tumour or retarded abnormal development.

4 Dense cellular white matter is due either to a residue of the last migratory wave with cells arranged in wave or columns or the glial proliferation preceding myelin deposition. Both features occur precisely where and when the common perinatal asoxic ischaemic lesions appear and thus may obscure or simulate such lesions.

Against this background some maldevelopmental processes will be discussed.

Migratory embryonal cells may be arrested and persist to form heterotopias. These may be islands of relatively well differentiated gray matter or clusters of cells with varying degree of differentiation. They form periventricularly (from matrix) in the white matter (from migratory waves) or the molecular layer (from the SGL). The periventricular islands may be mistaken for parts of basal ganglia, those closer to

the cortex as deep parts of gyri. Scattered heterotopic cells subcortically should be differentiated from normally occurring small numbers of subcortical nerve cells particularly occipitally. SGL remnants may project into the meninges containing neurons. The functional importance of heterotopias is unknown but they serve as indicator of arrested migration and concurrence of cortical malformation based on disturbed migration.

The graver cortical malformations agyria and pachygyria are easy to discover and often combined with extensive heterotopias.

The milder forms micropolygyria and mild dysplasia have structural abnormalities in common which are discussed under mild cortical dysplasia.

Characteristic of micropolygyria are areas of abnormally small closely packed gyri without scarring and with normal consistency. The gyri often are fused and may show 4 or 6 laminae with varying width. Malformed areas often are symmetrically distributed.

Mild cortical dysplasia, the most common cortical malformation presents diagnostic difficulties. Grossly the brain appears normal or shows some heterotopias and a suspicion of abnormally wide irregularly outlined cortex. Microscopically the malformation is of varying following abnormalities:

(a) abnormal cortical organization with poor to non-existent lamination, varying cell density, abnormally placed or oriented neurons.

(b) abnormal cortical maturation with poor differentiation of nerve cells, few dendrites and retarded or abnormal cortical myelination. Degenerative changes may add to the confusion of the cortical architecture.

(Supported by Medical Research Council grant B68 12X 2037-02.)

Edith Reake Nielsen & Helmer Søgaard (Aarhus, Denmark) *Neuropathological changes in epidermolysis bullosa congenita dystrophica*



## PROCEEDINGS OF PAEDIATRIC SOCIETIES

### SCANDINAVIAN SOCIETY OF PAEDIATRIC PATHOLOGY

Meeting in Copenhagen Denmark June 12-14 1969

Aagot Christie Løken (Oslo Norway) *Poliodystrophia cerebri progressiva infantilis*

*Poliodystrophia cerebri progressiva infantilis* is one name for a cerebral degeneration characterized by a diffuse loss of neurons throughout the cortex with a corresponding reactive astrogliosis. Involved to a lesser degree are the cerebellar cortex, the thalamic nuclei, the striatum and other nuclei. A progressive atrophy involving the white matter as well follows in prolonged cases.

The condition is seen in children who after a normal pregnancy and an uncomplicated delivery and postnatal period behave normally until a change in psychomotor development follows. There is a regression often complicated with jerks, rigidity and involuntary movements. Convulsions often complicate the picture until the child dies decerebrated in opisthotonus. The condition lasts from weeks to months or years. Two groups can be distinguished: one infantile with symptoms starting before 1 year, lasting up to 3 years of age; the second or juvenile with an onset between 3 and 6 years, lasting up to adult age.

**Case 1** A boy with a long history of vomiting and obstipation from 2-3 weeks of age gradually showed psychomotor retardation. Convulsions were at no time observed. He was hospitalized for malabsorption when he was 4 months old. He was treated in a home for mentally retarded children and was readmitted to hospital under the diagnosis of gastroenteritis shortly before he died 2 years and 7 months old. At autopsy there were no macro-

scopical changes in the viscera. The brain weighed 1160 g, appeared edematous with a moderately enlarged ventricular system and a thin corpus callosum. Extensive neuronal loss was found all through the cortex with astroglial proliferation and small sudanophilic microglia. Similar changes occurred in the striatum and globus pallidus. In the cerebellum there was loss of Purkinje cells and fibrillary gliosis. Some demyelination was seen in the temporal lobe.

**Case 2** A 17 month old boy with an unremarkable history until 16 months old when he had a cold with fever. The same day he had tonic and clonic convulsions and was admitted in hospital where he became comatose. EEG showed a diffuse mostly right sided dysrhythmia. He became decerebrated and died after 4 weeks. The histopathological changes were very similar to those reported in the first case except that the temporal lobes and hippocampus were spared.

The etiology was discussed. Anoxia is usually suspected although the first case gave no clinical indication for this. Familial occurrence has been reported. Of other possibilities were mentioned defective fetal development, metabolic disorders, infections, virus, lack of vitamin B<sub>1</sub>.

It was concluded that although anoxia could probably produce a similar picture, there are several reasons for suspecting some other primary factor. It is also possible that various metabolic or other pathogenetic mechanisms may result in similar morphological pictures.

flant according to the following histological features: vascular nuclei, presence of nuclei, presence of cytoplasm, presence of capsule, necroses and calcification as well as the presence of mature ganglion cells.

The importance of the first signs of histological differentiation in the tumor tissue was briefly discussed.

T. Normann & B. Otnes (Oslo, Norway): *Diffuse intestinal ganglioneuromatosis associated with tumors of endocrine organs*

A case of diffuse ganglioneuromatosis associated with mucosal neuromas, medullary thyroid carcinoma and severe diarrhea is reported in a 15 year old boy. To our knowledge only 4 cases with similar pathological changes in the intestine have been reported previously. In 3 of these the intestinal lesion was associated with a medullary thyroid carcinoma, mucosal neuromas, bilateral pheochromocytomas and severe diarrhea. Thick eyelids and lips gave these 3 and our own patient a peculiar facial similarity. In one of the previously reported cases a positive family history was obtained. In our patient the family history was negative. No pheochromocytomas have yet been diagnosed in our patient, but on one occasion slightly elevated values of urine catecholamine excretion was found.

The diffuse ganglioneuromatosis was usually thought to be the cause of the diarrhoea. However, intestinal passage time did not decrease satisfactorily after colectomy. After removal of his medullary thyroid carcinoma the maximal passage time showed a marked increase and subsequent loss a marked decrease. Several biologically active substances such as serotonin, prostaglandin and calcitonin have recently been found in medullary thyroid tumours. One of these might be responsible for the diarrhea seen in these patients. Diarrhea is also a prominent symptom in some patients with medullary thyroid carcinoma without associated tumours.

The similarity of lesions reported in these 4 patients indicates that this is not a random combination of unusual conditions but a syndrome. This is probably related to the more common often familiarly occurring combination of medullary thyroid carcinoma and bilateral pheochromocytomas.

(Submitted for publication in Scandinavian Journal of Gastroenterology)

Edith Reske Nielsen, H. Sogaard & Aa. Harmsen (Aarhus, Denmark): *A case of genetically allied congenital toxoplasmosis*

Three weeks after a normal birth an infant developed somnolence and generalized rigidity. The cranium was hydrocephalic and the fontanelles were tense. The CSF contained 1800 mg. protein but no cells. There were large grayish opacities in the vitreous. The X ray of the skull showed scattered intracranial calcifications. Her temperature was periodically raised and she had repeated generalized tonic fits. She died 40 days old.

The normally developed brain weighed after fixation 515 g. The leptomeninges were thickened and discolored and there were large yellowish depressed and softened cortical areas. In the coronal sections there were necrotic and cystic regions and the walls of the ventricular system were thickened in some places, necrotic in others. The spinal cord was swollen. Both eyes showed severe pathological changes with exudates, retinal detachment, sclerosis and thickening of the retina and the choroid and opacity of the vitreous.

The leptomeninges, the brain and the spinal cord contained granulomatous areas often with a necrotic centre. Between proliferating capillaries and fibroblasts were many phagocytes, lymphocytes and a few plasma cells and eosinophilic leucocytes. The macrophages contain numerous typical toxoplasma and many pseudocysts are present. In both the brain and the spinal cord there were places where the inflammatory reaction was diffuse and others where there were toxoplasma without reaction.

Two cases (age 9 and 15 years) of epidermolysis bullosa congenita dystrophica are reported.

The characteristic macroscopical and microscopical changes in the epidermis and corium were found but in addition with vascular lesions with intima thickening and calcification of all the layers. In both cases a severe generalized amyloidosis had developed and the children died in uremia. The peripheral nerves had signs of demyelination and in one of the cases a severe clear-cut degeneration with loss of the myelin sheaths of the posterior columns was seen. These changes have not been reported previously.

A reasonable hypothesis is that the lesions of the skin and the nervous system are caused by a common ectodermal embryonal defect.

H. Sogaard & K. Bertelsen (Aarhus, Denmark). *Developmental tumors of the base of the skull in newborn infants*

Four cases of benign congenital teratoma in newborn infants are reported. In two cases the tumor arose from the base of the skull, filled the entire buccal cavity and protruded from the mouth. In another case the tumor was present occipitally and was associated with anencephaly, and in the fourth case the tumor was found in relationship with the tonsils.

The patients were operated on; three are still alive (five months—two years after the operation). Incomplete development of the brain was seen in only one case, and in contrast to other published cases of similar cases no congenital malformations were found in other parts of the body.

The histological pattern of the tumors is verified by illustrations and the origin discussed.

Judit Makinen (Helsinki, Finland). *Neuroblastoma. Histological classification of 54 cases*

Fifty-four cases of neuroblastoma in children 0–14 years old were treated between the years

1950 and 1966 in the Children's Hospital University of Helsinki.

Thirty-two of these patients died within the first six months and nine within the second months after the diagnosis was made and treatment was started. The 13 children who survived at least one year were alive and without clinical evidence of disease in March 1969.

The shortest follow-up was three and a half years; the longest 13 and a half years.

The histological features of the biopsy and/or autopsy specimens taken from the primary tumor were retrospectively examined in all cases in an attempt to find significant differences between the fatal and non-fatal cases.

The following microscopic features were evaluated, either as indicating differentiation toward ganglioneuroma or as a better prognostic sign as reported by several authors:

- 1 Increase in nuclear size with the presence of pale, spherical, often vesicular nuclei
- 2 Nucleoli
- 3 Slightly acidophilic cytoplasm
- 4 Cytoplasmic cell processes
- 5 Appearance of fibrillary intracellular material
- 6 Ball-like clumping of the tumor cells
- 7 Presence of true rosette formations
- 8 Presence of fibrous capsule
- 9 Hemorrhages in the tumor tissue
- 10 Necroses in the tumor tissue
- 11 Calcification in the tumor tissue
- 12 Presence of ganglion cells

Histologically the tumors were classified into the following groups:

Group I—no histological sign of differentiation

Group II—various signs of histological differentiation

The second group was divided into two parts:

Group II a—without ganglion cells

Group II b—with the presence of completely differentiated ganglion cells

In the statistical analysis the difference between the fatal and non-fatal cases was significant.

ficant according to the following histological features: vesicular nuclei, presence of nucleoli, presence of cytoplasm, presence of capsule, necroses and calcification as well as the presence of mature ganglion cells.

The importance of the first signs of histological differentiation in the tumor tissue was briefly discussed.

T. Normann & H. Ottesen (Oslo, Norway) *Diffuse intestinal ganglioneuromatosis associated with tumors of endocrine organs*

A case of diffuse ganglioneuromatosis associated with mucosal neuromas, medullary thyroid carcinoma and severe diarrhea is reported in a 15 year old boy. To our knowledge only 4 cases with similar pathological changes in the intestine have been reported previously. In 3 of these the intestinal lesion was associated with a medullary thyroid carcinoma, mucosal neuromas, bilateral pheochromocytomas and severe diarrhea. Thick eyelids and lips gave these 3 and our own patient a peculiar facial similarity. In one of the previously reported cases a positive family history was obtained. In our patient the family history was negative. No pheochromocytomas have yet been diagnosed in our patient, but on one occasion slightly elevated values of urine catecholamine excretion was found.

The diffuse ganglioneuromatosis was initially thought to be the cause of the diarrhoea. However, intestinal passage time did not decrease satisfactorily after colectomy. After removal of his medullary thyroid carcinoma the intestinal passage time showed a marked increase and thyrotoxicosis, a marked decrease. Several biologically active substances such as serotonin, prostaglandin and calcitonin have recently been found in medullary thyroid tumours. One of these might be responsible for the diarrhea seen in these patients. Diarrhea is also a prominent symptom in some patients with medullary thyroid carcinoma without associated lesions.

The similarity of lesions reported in these 4 patients indicates that this is not a random combination of unusual conditions but a syndrome. This is probably related to the more common, often familiarly occurring combination of medullary thyroid carcinoma and bilateral pheochromocytomas.

(Submitted for publication in Scandinavian Journal of Gastroenterology)

Edith Renke Nielsen, H. Sjøgaard & Aa. Harmsen (Aarhus, Denmark) *A case of genetically altered congenital toxoplasmosis*

Three weeks after a normal birth an infant developed somnolence and generalized rigidity. The cranium was hydrocephalic and the fontanelles were tense. The CSF contained 1800 mg. protein but no cells. There were large grayish opacities in the vitreous. The X ray of the skull showed scattered intracranial calcifications. Her temperature was periodically raised and she had repeated generalized tonic fits. She died 40 days old.

The normally developed brain weighed after fixation 515 g. The leptomeninges were thickened and discolored and there were large yellowish, depressed and softened cortical areas. In the coronal sections there were necrotic and cystic regions and the walls of the ventricular system were thickened in some places, necrotic in others. The spinal cord was swollen. Both eyes showed severe pathological changes with exudative retinal detachment, sclerosis and thickening of the retina and the choroid and opacity of the vitreous.

The leptomeninges, the brain and the spinal cord contained granulomatous areas, often with a necrotic centre. Between proliferating capillaries and fibroblasts were many phagocytes, lymphocytes and a few plasma cells and eosinophilic leucocytes. The macrophages contain numerous typical toxoplasma and many of the endocysts are present. In both the brain and the spinal cord there were places where the inflammatory reaction was diffuse and others where there were toxoplasma without reaction.

Two cases (age 9 and 15 years) of epidermolysis bullosa congenita dystrophica are reported

The characteristic macroscopical and microscopical changes in the epidermis and corium were found but in addition with vascular lesions with intima thickening and calcification of all the layers. In both cases a severe generalized amyloidosis had developed and the children died in uremia. The peripheral nerves had signs of demyelination and in one of the cases a severe clear-cut degeneration with loss of the myelin sheaths of the posterior columns was seen. These changes have not been reported previously.

A reasonable hypothesis is that the lesions of the skin and the nervous system are caused by a common ectodermal embryonal defect.

H. Sogaard & K. Bertelsen (Aarhus, Denmark) *Developmental tumors of the base of the skull in newborn infants*

Four cases of benign congenital teratoma in newborn infants are reported. In two cases the tumor arose from the base of the skull, filled the entire buccal cavity and protruded from the mouth. In another case the tumor was present occipitally and was associated with anencephaly and in the fourth case the tumor was found in relationship with the tonsils.

The patients were operated on; three are still alive (five months—two years after the operation). Incomplete development of the brain was seen in only one case and in contrast to other published series of similar cases no congenital malformations were found in other parts of the body.

The histological pattern of the tumors is verified by illustrations and the origin discussed.

Judit Makinen (Helsinki, Finland) *Neuroblastoma. Histological classification of 54 cases*

Fifty-four cases of neuroblastoma in children 0–14 years old were treated between the years

1950 and 1966 in the Children's Hospital, University of Helsinki.

Thirty-two of these patients died within the first six months and nine within the second six months after the diagnosis was made and treatment was started. The 13 children who survived at least one year were alive and without clinical evidence of disease in March 1969.

The shortest follow-up was three and a half years; the longest 13 and a half years.

The histological features of the biopsy and/or autopsy specimens taken from the primary tumor were retrospectively examined in all cases in an attempt to find significant difference between the fatal and non-fatal cases.

The following microscopical features were evaluated either as indicating differentiation toward ganglioneuroma or as a better prognostic sign as reported by several authors:

- 1 Increase in nuclear size with the presence of pale, spherical, often vesicular nuclei
- 2 Nucleoli
- 3 Slightly acidophilic cytoplasm
- 4 Cytoplasmic cell processes
- 5 Appearance of fibrillary intracellular material
- 6 Ball-like clumping of the tumor cells
- 7 Presence of true rosette formations
- 8 Presence of fibrous capsule
- 9 Hemorrhages in the tumor tissue
- 10 Necroses in the tumor tissue
- 11 Calcification in the tumor tissue
- 12 Presence of ganglion cells

Histologically the tumors were classified into the following groups:

Group I—no histological sign of differentiation

Group II—various signs of histological differentiation

The second group was divided into two parts:

Group II a—without ganglion cells

Group II b—with the presence of completely differentiated ganglion cells

In the statistical analysis the difference between the fatal and non-fatal case was significant.

B Robertson & B I Ivermark (Stockholm Sweden) *Abnormalities of the costochondral junction in cases of perinatal death with special reference to hyaline membrane disease*

Microscopic examination of decalcified sections from the costochondral junction in a consecutive series of 70 neonatal autopsy subjects revealed changes in 76 per cent. In 32 per cent the lesion was non specific and termed chondral dysplasia most of these instances being only slightly affected. In 22 per cent the metaphysis had a porous appearance displaying osteoclasts reduced hematopoietic activity and hypovascularity giving the area the appearance of trabecular rarefaction. A third group (22 per cent) had an intermediate type of change. In altogether 58 per cent of the abnormal specimens the abnormal metaphyseal zone was demarcated clearly visible in low power fields and measuring 500-1500 microns. The size of this reactive zone was not correlated to the degree of prematurity or to the time of postnatal survival. However the presence of this zone showed a marked correlation to the occurrence of pulmonary hyaline membranes suggesting a common etiologic factor operating several days before birth.

#### References

- 1 Emery J L & Halpern P A. *Arch Dis Child* 42: 1 1967
- Robertson B & Ivermark B I. *Acta Path Microbiol Scand*. In press

H Sogaard K W Kastrup & Edith Recke Nielsen (Aarhus Denmark) *Hydranencephaly*

The clinical features in a girl with hydranencephaly is reported. The pregnancy was uncomplicated. The child was born as the second in a pair of twins. The twin brother is healthy. Respiratory distress developed in the first 24 hours. The child failed to thrive and developed signs of internal hydrocephalus. Death occurred at the age of 4 months. Neurological examination demonstrated persistence of neonatal automatisms including grasp Moro and stepping reflexes. There was loss of vision. EEG

showed absence of electrical activity over the major part of the brain. Echoencephalograms failed to demonstrate ventricular echoes. In the pneumoencephalograms there was a free floating airbubble. Transillumination of the head caused it to glow red all over and was found to be a simple test in confirming the diagnosis as did a decreased skin temperature on the skull. Poikilothermia was a prominent feature.

Spontaneous motonic function decreased and as the condition deteriorated an increased flexoronus was observed with twitching spasms. Intrauterine infection with toxoplasma and cytomegalic virus was ruled out.

The loss of distal phalanges on 2 fingers and a systolic murmur indicating a septal defect was also observed. Isostenuria and inability to increase the osmolality of the urine in a salt loading test (Cotlet) was observed. Injection of antidiuretic hormone corrected this and there by confirmed the diagnosis of diabetes insipidus.

The glucose tolerance test was normal and also the insulin provocative test. Determination of secretion of growth hormone as stimulated by hypoglycemia revealed a normal response suggesting the persistence of at least some function in the anterior pituitary lobe.

Angiography showed a closure of the internal carotid vessels just below the entrance into the skull and persistence of a functioning vertebral basilar system supporting the theory of a possible insufficiency as an etiological factor.

#### Autopsy

Four month old girl weight 4350 gr length 55 cm. The second and third left finger had no distal phalanges but other external congenital malformation was not observed. The cranium with moderate hydrocephalus measured 47 cm. A congenital heart malformation was found consisting of valvular aortic stenosis septum primum defect hypertrophy of the right ventricle and a persistent ductus arteriosus but no other congenital malformations were observed in the organs. The adrenal glands were normal.

The cranial cavity contained 300 cc xantho-

Many ureters were encrusted with calcium salts. There were severe degenerative changes of the ganglion cells and nerve fibres throughout the central nervous system. Around miliary granulomas there were swollen astrocytes and perivascular infiltrations of lymphocytes. Both eyes had severe chorioretinitis with destruction and necrosis of the retina and many toxoplasma in cysts.

Dorthe Francis & J. C. Melchior (Copenhagen Denmark) *Toxoplasmosis acquisita* = case report

Toxoplasmosis acquisita is usually a benign infection. Only a few fatal cases have been reported. Sabin (1), Pinkerton & Henderson (2) and Sexton *et al.* (3) and the patients in question died from acute primary infection.

The following case is reported because of an unusually long history of acquired toxoplasmosis terminating in death after a minor operation.

#### Case report

An eight year old boy was admitted to hospital with abdominal distension. Four years previously he had been hospitalised for acquired toxoplasmosis diagnosed on the basis of a high toxoplasmosis neutralisation test (1:1250), a positive culture from lymphnodes and histological changes in the same lymphnodes similar to those caused by toxoplasmosis. Subsequently he had enlargement of lymphnodes, abdominal distension with hepatomegaly, periods of fever, enteritis and atypical pneumonia, chronic sinusitis and middle ear infection. Adenectomy was carried out without immediate complications. After a few days of raised temperature however he suddenly developed hyperpyrexia, shock and died within a few hours.

Laboratory examinations were within normal limits except for the very low gamma globulin fraction of the plasmoproteins.

No pathogenic bacteria were isolated from blood tissue (lymphnodes), urine or feces, cultures for *M. tuberculosis* were also negative.

The autopsy findings were a firm atelectasis in the right lung, hepatomegaly and multiple ulcerations in the intestinal tract with fibrin on the peritoneal surface and fibrous adhesions. The pancreas was fibrotic with retroperitoneal fibrosis occluding the inferior vena cava and right ureter. There was generalized lymph node enlargement. The thymus could not be detected in the mediastinum. The central nervous system was macroscopically normal.

Microscopically there were chronic interstitial pneumonia and slight lymphocyte infiltration of the myocardium and the liver. In the intestinal tract and the lymphnodes there were changes in the lymphatic tissue with fibrosis, marked sinus and follicular reticulosis, small necroses and numerous large macrophages with PAS positive granules in the cytoplasm. In the pancreas and the retroperitoneal space there were fibrosis and pronounced infiltration with lymphocytes and plasma cells. Most of the glandular tissue in the pancreas had disappeared leaving irregular insulae and a few normal ducts. In the mediastinum small islands of hypoplastic thymic tissue were seen. In the brain there were slight and diffuse degeneration of ganglion cells, gliosis and lymphatic infiltration. A single small necrosis was observed incorporating a circular structure about 20  $\mu$  in diameter and containing numerous granules somewhat similar to toxoplasma bodies.

At autopsy no pathogenic bacteria including *M. tuberculosis* were isolated from blood tissue or feces and toxoplasma organisms were cultivated only from the brain.

The unusual course of acquired toxoplasmosis in this boy and his sudden death after a minor operation may be explained as a result of an immunological deficiency state causing the severe widespread chronic inflammation.

#### References

1. Sabin A. B. *JAMA* 116: 801 1941
2. Pinkerton H. & Henderson E. G. *JAMA* 116: 807 1941
3. Sexton E. C., Eyles D. E. & Dellman R. E. *Am J Med* 14: 366 1953

it is suggested in early phase of sulfatidoses to treat the patient with a vitamin A deficient diet (1).

The lack of  $\beta$  D galactosidase was found in the kin of three Greenland children (Table 1) clinically characterized by a slightly increased excretion of AMP base defects—especially in renal shaga as verified by X ray examination—and by the clinical finding of co-sanguinity of the parents (2-5). The lack of  $\beta$  D galactosidase in these case supplements van Hooft & Hers (10) list of mucopolysaccharidoses with known lysosomal defects (lysosis dosi generalized galactosidosis monosaccharidoses, selective lack of galactosidase in several but not all organs and the elevated galactosidase disorder). The data also agree with those of Chickerman & Kohlin (11).

## References

1. Claeson J. *European J Biochem* 7:575 1969
2. Claeson J. Dyggve H V & Melchior J C. *Arch Dis Child* 38:364 1963
3. Claeson J. Melchior J C & Dyggve H V. *Arch Dis Child* (to be published)
4. DeDuve G. *The Lysosome Concept*. Ciba Symp on Lysosomes Churchill Ltd London 1963 pp 1-31
5. Dyggve H V. Melchior J C & Claeson J. *Arch Dis Child* 37:515 1962
6. Low Christensen H O & Claeson J. *Clin Chem Acta* 18:465 1967
7. Melchior J C & Claeson J. *Acta Paed et Scand* 57:2 1967
8. Nordhoff A B. *Lysosomes in the physiology and pathology of cells*. Ciba Symp on Lysosomes Churchill Ltd London 1966 p 36
9. van Hooft F & Hers H O. *Rev Internat Hepatol* 8:815 1967
10. —. *European J Biochem* 7:34 1968
11. Chickerman P A & Kohlin P. *Acta Paed et Scand* 57:181 1968

Torben Schrødt & K. Mannitzén (Copenhagen Denmark). *Hepatoblastoma and hepatocarcinoma in infancy and childhood*

The need for a reliable histological classification of malignant tumors of the liver in infancy has increased during the last years since the results of surgical treatment of hepatoblasto-

mas are by far much better than those of hepatocarcinomas.

A series of 11 cases of hepatoblastomas and one case of hepatocarcinoma in children admitted to the Rigshospitalet of Copenhagen during the last 10 years is presented.

## Hepatoblastomas

**Age and sex.** 5 of the cases were in boys and one in a girl all ranging in age from two months to two and a half years.

**Site localization and gross anatomy.** The average size was about 10 cm in diameter except in the case of the boy of two months whose tumor measured 3 cm. The tumors were localized to the right liver lobe except in one case. They appeared grossly lobular and more or less encapsulated.

**Classification.** After Willis the tumors were divided in pure epithelial and mixed i.e. tumors with an epithelial as well as a mesenchymal component.

**Microscopic appearance.** In both epithelial and mixed tumors two epithelial types were encountered.

(a) **Embryonal type** with very little cellular differentiation corresponding to the very early phase of liver embryogenesis. The cells were small dark with a high mitotic rate and often arranged in rosette formation. Extramedullary hemopoiesis was not found in the areas of this cellular type.

(b) **Fetal type** had the appearance of the pre-natal liver tissue arranged in liver plates. The cells were rather light with a lesser nucleus cytoplasm ratio than the former type. Extramedullary hemopoiesis was often encountered.

The mesenchymal component of the mixed type consisted ordinarily of loose and primitive connective tissue or of osteoid like tissue whereas cartilage was not found.

## Hepatocarcinoma

This tumor was diagnosed in only one case in a 14 year old girl. The histological picture was



Table 1 Lysosomal enzymic activities of dermal biopsies<sup>1</sup>Activity  $\times 10^{-3}$  measured in  $\mu$ moles/min/mg protein

Dermal biopsies Lysosomal enzymes	MJHJ	HSOMJ	LAAAJ	Normal mean
<i>Substrates</i>				
1 <i>p</i> Nitrophenyl $\alpha$ -D-murino pyranosidase	2.69	3.34	3.97	0.84 $\pm$ 0.53
2 <i>p</i> Nitrophenyl N-acetyl $\beta$ -D-glucosaminidase	25.7	30.61	17.29	24.9 $\pm$ 2.6
3 <i>p</i> Nitrophenyl $\beta$ -D-galacto-pyranosidase	0	0	0	7.1 $\pm$ 1.0
4 <i>p</i> Nitrophenyl $\alpha$ -L-fuco pyranosidase	2.66	8.45	6.92	15.7 $\pm$ 3.1
5 Nitro phosphate	75.33	124.95	70.3	156 $\pm$ 11
6 <i>p</i> Nitrophenyl $\beta$ -D-glucuronidase	1.77	4.22	4.61	5.6 $\pm$ 0.4

<sup>1</sup> Cf. Clausen J, Melchior J C & Dyggve H V (1969)

chrome cerebro spinal fluid & normally developed fetal cerebri and tentorium cerebelli with scattered hemorrhages. Only the basal parts of the temporal lobes, the hippocampus and the eyes were present of the procerebrum. The cerebellum, the brain stem and the cranial nerves were normal. It was possible to identify most of the cerebral arteries in the thin layer of cerebral tissue at the base of the middle cerebral fossa. In the sella turcica a small hypophysis was found but only the adenohypophysis was intact, the neurohypophysis was totally replaced by collagenous tissue. The pathogenesis of this peculiar malformation was discussed and the case documented by illustrations.

Jørgen Clausen, Johannes C. Melchior & Holger V. Dyggve (Copenhagen, Denmark)  
*Progressive encephalopathies*

The original classification of progressive encephalopathies associated with storage of polar lipids and/or polysaccharides has been based on the symptomatology and the type of chemical material stored. In using this classification certain clinical entities, e.g. the mucopolysaccharidoses, showed overlapping chemical abnormalities. Excretion of acid mucopolysaccharides (AMP) in these diseases involved excretion of chondroitin sulfate B alone or associated with excretion of keratosulfate and/or heparin sulfate. Furthermore, these entities similar to the infantile form of gangliosidosis

were often seen in connection with storage of mono-sialoganglioside in the central nervous system.

After the discovery of the lysosomes (4, 8) and after the demonstration that the so-called thesaurocytes found in Hurler's syndrome are identical with swollen lysosomes (9), it has been possible to classify lipidoses and mucopolysaccharidoses as lysosomal disorders with lack of a lysosomal enzyme degrading the stored material.

The compounds with the highest colloid content, e.g. tri-sialogangliosides (possibly protein bound) and AMP, protein bound by a carbohydrate bridge of one or more monosaccharides (fucose, mannose, arabinose) are degraded by a stepwise liberation of sialic acid, saccharides and protein. The degradation is catalyzed by lysosomal enzymes. Corresponding to most of the enzymic steps, disorders have been characterized by lack of the corresponding enzyme.

Of the disorders of lysosomal pathogenesis, sulfatidosis (lack of sulfatide sulfatase) and lack of  $\beta$ -D-galactosidase are demonstrated in the present communication (cf. 3, 7). Sulfatidosis was biochemically traced in family studies by quantitative thin layer chromatography (6) of lipids extractable from urinary sediment and serum and by enzymic determination of lysosomal enzymes. These methods revealed genetic carriers of the disease. Since vitamin A is necessary for the formation of active sulfate (PAPS)—one of the precursors of sulfatide—

it is suggested in early phase of sulfatidoses to treat the patient with a vitamin A deficient diet (1).

The lack of  $\beta$  D galactosidase was found in the kin of three Greenland children (Table 1) clinically characterized by a slightly increased excretion of AMP base defects—especially in uric acid—as verified by X ray examination—and by the clinical finding of co-sanguinity of the parents (2-5). The lack of  $\beta$  D galactosidase in these case, supplements van Hoof & Hers (10) list of mucopolysaccharidoses with known lysosomal defects (lucosidosis, generalized galactosidosis, monosaccharidosis, elective lack of galactosidase in several but not all organs and the elevated galactosidase disorder). The results also agree with those of Ockerman & Kohnen (11).

## References

1. Clausen J. *Europ J Biochem* 7: 473 1969
2. Clausen J, Dyggve H V & Melchior J C. *Arch Dis Child* 38: 364 1963
3. Clausen J, Melchior J C & Dyggve H V. *Arch Dis Child* (no in published)
4. DeDuve G. The Lysosome Concept. Ciba Symp on Lysosomes. Churchill Ltd. London 1963 pp 1-31
5. Dyggve H V, Melchior J C & Clausen J. *Arch Dis Child* 37: 525 1962
6. Lou Christensen H O & Clausen J. *Clin Chim Acta* 18: 445 1967
7. Melchior J C & Clausen J. *Acta Paediat Scand* 57: 2 1967
8. Horekoff A B. Lysosomes in the physiology and pathology of cells. Ciba Symp on Lysosomes. Churchill Ltd. London 1963 p 36
9. van Hoof F & Hers H G. *Re Internat Hepatol* 4: 815 1967
10. —. *Europ J Biochem* 7: 34 1968
11. Ockerman P A & Kohnen P. *Acta Paediat Scand* 57: 281 1968

Torben Schudt & K. Maustitzen (Copenhagen Denmark). *Hepatoblastoma and Hepatocarcinoma in infancy and childhood*

The need for a reliable histological classification of malignant tumors of the liver in infancy has increased during the last years since the results of surgical treatment of hepatoblasto-

mas are by far much better than those of hepatocarcinomas.

A series of 6 cases of hepatoblastomas and one case of hepatocarcinoma in children admitted to the Rigshospitalet of Copenhagen during the last 10 years is presented.

## Hepatoblastomas

**Age and sex.** 5 of the cases were in boys and one in a girl, all ranging in age from two months to two and a half years.

**Site localization and gross anatomy.** The average size was about 10 cm in diameter except in the case of the boy of two months whose tumor measured 3 cm. The tumors were localized to the right liver lobe except in one case. They appeared grossly lobular and more or less encapsulated.

**Classification.** After Willis the tumors were divided in pure epithelial and mixed i.e. tumors with an epithelial as well as a mesenchymal component.

**Microscopic appearance.** In both epithelial and mixed tumors two epithelial types were encountered:

(a) **Embryonal type** with very little cellular differentiation corresponding to the very early phase of liver embryogenesis. The cells were small, dark, with a high mitotic rate and often arranged in rosette formation. Extramedullary hemopoiesis was not found in the areas of this cellular type.

(b) **Fetal type** had the appearance of the pre-natal liver tissue arranged in liver plates. The cells were rather light with a lesser nucleus/cytoplasm ratio than the former type. Extramedullary hemopoiesis was often encountered.

The mesenchymal component of the mixed type consisted ordinarily of loose and primitive connective tissue or of osteoid like tissue whereas cartilage was not found.

## Hepatocarcinoma

The tumor was diagnosed in only one case in a 14 year-old girl. The histological picture was

Table 1 *Lysosomal enzymic activities of dermal biopsies<sup>1</sup>*Activity  $\times 10^{-4}$  measured in  $\mu$ moles/min/mg protein

Dermal biopsies				
Lysosomal enzymes	M J H J	H S O M J	L A A A J	Normal mean
<i>Substrates</i>				
1 <i>p</i> Nitrophenyl $\alpha$ D manno pyranosidase	2.69	6.34	3.97	0.84 $\pm$ 0.53
2 <i>p</i> Nitrophenyl N acetyl $\beta$ D glucosamidase	25.7	30.61	17.29	28.9 $\pm$ 2.5
3 <i>p</i> Nitrophenyl $\beta$ D galacto pyranosidase	0	0	0	7.1 $\pm$ 1.0
4 <i>p</i> Nitrophenyl $\alpha$ L fucosidase	2.66	8.45	6.92	15.7 $\pm$ 3.1
5 <i>p</i> Nitro phosphatase	75.33	124.95	70.3	156 $\pm$ 11
6 <i>p</i> Nitrophenyl $\beta$ D glucuronidase	1.77	4.22	4.61	5.6 $\pm$ 0.4

<sup>1</sup> Cf Clausen J Melchior J C & Dyggve H V (1969)

chrome cerebrospinal fluid a normally developed falx cerebri and tentorium cerebelli with scattered hemorrhages. Only the basal parts of the temporal lobes, the hippocampus and the eyes were present of the procencephalon. The cerebellum, the brain stem and the cranial nerves were normal. It was possible to identify most of the cerebral arteries in the thin layer of cerebral tissue at the base of the middle cerebral fossa. In the sella turcica a small hypophysis was found but only the adenohypophysis was intact; the neurohypophysis was totally replaced by collagenous tissue. The pathogenesis of this peculiar malformation was discussed and the case documented by illustrations.

Jørgen Clausen, Johannes C. Melchior & Holger V. Dyggve (Copenhagen, Denmark)  
*Progressive encephalopathies*

The original classification of progressive encephalopathies associated with storage of polar lipids and/or polysaccharides has been based on the symptomatology and the type of chemical material stored. In using this classification certain clinical entities, e.g. the mucopolysaccharidoses, showed overlapping chemical abnormalities. Excretion of acid mucopolysaccharides (AMP) in these diseases involved excretion of chondroitin sulfate II alone or as associated with excretion of keratosulfate and/or heparin sulfate. Furthermore, these entities similar to the infantile form of gangliosidosis

were often seen in connection with storage of mono sialoganglioside in the central nervous system.

After the discovery of the lysosomes (4, 8) and after the demonstration that the so-called thesaurocytes found in Hurler's syndrome are identical with swollen lysosomes (9), it has been possible to classify lipidoses and mucopolysaccharidoses as lysosomal disorders with lack of a lysosomal enzyme degrading the stored material.

The compounds with the highest colloid content, e.g. tri sialogangliosides (possibly protein bound) and AMP, protein bound by a carbohydrate bridge of one or more monosaccharides (fucose, mannose, arabinose) are degraded by a stepwise liberation of sialic acid, saccharides and protein. The degradation is catalyzed by lysosomal enzymes. Corresponding to most of the enzymic steps, disorders have been characterized by lack of the corresponding enzyme.

Of the disorders of lysosomal pathogenesis, sulfatidosis (lack of sulfatide sulfatase) and lack of  $\beta$ -D galactosidase are demonstrated in the present communication (cf 3, 7). Sulfatidosis was biochemically traced in family studies by quantitative thin layer chromatography (6) of lipids extractable from urinary sediment and serum and by enzymic determination of lysosomal enzymes. These methods revealed genetic carriers of the disease. Since vitamin A is necessary for the formation of active sulfate (PAPS)—one of the precursors of sulfatide—

it is suggested in early phase of sulfatidoses to treat the patient with a vitamin A deficient diet (1).

The lack of  $\beta$  D galactosidase was found in the kin of three Greenland children (Table 1) clinically characterized by a slightly increased excretion of AMP base defects—especially in uric acid as verified by X ray examination—and by the clinical finding of cyananginity of the parents (2-5). The lack of  $\beta$  D galactosidase in these case supplements van Hoof & Hers (10) list of mucopolysaccharidoses with known lysosomal defects (fucosidosis, generalized galactosidosis, monosaccharidosis, elective lack of galactosidase in several but not all organs and the elevated galactosidase disorder). The data also agree with those of Ockerman & Kohlin (11).

## References

1. Claessen J. *European J Biochem* 7: 575 1969.
2. Claessen J., Dyggve H. V. & Melchior J. C. *Arch Dis Child* 38: 364 1963.
3. Claessen J., Melchior J. C. & Dyggve H. V. *Arch Dis Child* (to be published).
4. DeDroes G. The Lysosomes Concepts. Ciba Symp on Lysosomes. Churchill Ltd. London 1963 pp. 1-31.
5. Dyggve H. V., Melchior J. C. & Claessen J. *Arch Dis Child* 37: 525 1962.
6. Lou Christensen H. O. & Claessen J. *Clin Chim Acta* 18: 465 1967.
7. Melchior J. C. & Claessen J. *Acta Paediat Scand* 57: 2 1967.
8. Norrskott A. B. Lysosomes in the physiology and pathology of cells. Ciba Symp on Lysosomes. Churchill Ltd. London 1963 p. 34.
9. van Hoof P. & Hers H. O. *Rev. Internat. Hematol* 8: 815 1967.
10. —. *European J Biochem* 7: 34 1968.
11. Ockerman P. A. & Kohlin P. *Acta Paediat Scand* 57: 231 1968.

Torben Schrødt & J. Møntzen (Copenhagen, Denmark). *Hepatoblastoma and hepatocarcinoma in infancy and childhood*

The need for a reliable histological classification of malignant tumors of the liver in infancy has increased during the last years since the results of surgical treatment of hepatoblasto-

mas are by far much better than those of hepatocarcinomas.

A series of 6 cases of hepatoblastomas and one case of hepatocarcinoma in children admitted to the Rigshospitalet of Copenhagen during the last 10 years is presented.

## Hepatoblastomas

*Age and sex.* 5 of the cases were in boys and one in a girl, all ranging in age from two months to two and a half years.

*Site, localization and gross anatomy.* The average size was about 10 cm in diameter except in the case of the boy of two months whose tumor measured 3 cm. The tumors were localized to the right liver lobe except in one case. They appeared grossly lobular and more or less encapsulated.

*Classification.* After Willis the tumors were divided in pure epithelial and mixed i.e. tumors with an epithelial as well as a mesenchymal component.

*Microscopic appearance.* In both epithelial and mixed tumors two epithelial types were encountered.

(a) *Embryonal type* with very little cellular differentiation corresponding to the very early phase of liver embryogenesis. The cells were small, dark, with a high mitotic rate and often arranged in rosette formation. Extramedullary hemopoiesis was not found in the areas of this cellular type.

(b) *Fetal type* had the appearance of the pre-natal liver tissue arranged in liver plates. The cells were rather light with a lesser nucleus/cytoplasm ratio than the former type. Extramedullary hemopoiesis was often encountered.

The mesenchymal component of the mixed type consisted ordinarily of loose and primitive connective tissue or of osteoid-like tissue whereas cartilage was not found.

## Hepatocarcinoma

This tumor was diagnosed in only one case in a 14-year-old girl. The histological picture was

that of adult hepatoma with a well pronounced polymorphism and with many giant tumor cells

#### *Follow up*

Two cases of hepatoblastoma survived a surgical resection with a follow up of 24 months and 29 months respectively. Two died of cardiac arrest during operation, one died of the tumor two months after operation and one case was not followed but the patient was in poor condition when discharged from hospital.

The girl with hepatocarcinoma died of cardiac arrest during operation. There were metastases to the retroperitoneal lymph nodes.

One case of hepatoblastoma showed an *endocrine disorder*. On admission a boy one and a half years old with hepatomegaly had precocious puberty with pubic hair development and elevation of urinary gonadotrophins to 9 i.u. After surgical resection of the tumour microscopically a mixed hepatoblastoma the activity had fallen to less than three units. Half a year after the operation this normal value was maintained and the pubic hairs had disappeared. The boy was completely healthy one year after the operation.

#### Jørn Engel Møller (Copenhagen, Denmark) *Diagnostic value of cortex biopsy*

There has been increasing interest in cerebral cortical biopsy studies as the results obtained through this procedure are valuable for diagnosis, prognosis and research in neurology, neurosurgery, psychiatry and in related basic science.

As the post operative risks of infection, hemorrhage and epilepsy are negligible and as the technical laboratory possibilities are increasing the clinicians are more frequent interested in cortical biopsy examination as an adjunctive diagnostic procedure.

The term cortical biopsy is used even if it means a biopsy which includes an adequate amount of leptomeninges, cortical grey matter and subcortical white matter.

The literature on the subject is scarce.

As Biemond (1965) points out after collecting the opinions of experienced neurologists in Europe and USA the following criteria must be fulfilled regarding the types of disease in which cerebral biopsy may be considered:

- 1 The manifestations of the illness must clearly point at a diffuse cerebral disorder accompanied by dementia.
- 2 The course of the disease must be unmistakably progressive.
- 3 The clinical diagnosis must have remained uncertain despite employment of all indicated neurophysiological and clinical methods including serological, chemical and microscopical examination of fluids and in special cases including biopsies from muscles, peripheral nerves and rectum.

In the future the above criteria may have to be extended because evolution in biopsy studies outside the brain has shown that the indications are widened especially with regard to research.

It must always be remembered that the procedure involves the most precious of the human resources. For this reason it is assumed that the entire problem is discussed by the clinical staff, the surgeon, the pathologist, the chemist and the ultramicroscopist.

The biopsy has to be done with the patient in general anesthesia. It is very important that a block of brain tissue measuring 1 cm<sup>3</sup> to 1.5 cm<sup>3</sup> in size including the leptomeninges, cortex and subcortical white matter is non traumatically sharply excised.

Ventriculography can be carried out concurrently with the brain biopsy.

An optimal biopsy specimen is divided for the following examinations: Histological, histochemical, electronmicroscopical and biochemical study.

The present material consists of 180 cortical biopsies. Here the pre biopsy clinical diagnosis has been compared with the neuropathological diagnosis.

The biopsy was considered to be of value if

it either confirmed or made a new diagnosis. 138 of the 180 biopsies had histological abnormalities—40 showed inflammatory changes, 11 degenerative, 13 presenile senile and arterio sclerotic changes. In 11 cases developmental abnormalities were present. Two had aneurysms, 50 non specific degeneration and in 10 biopsies no definite diagnosis could be

given due to artefacts. 42 biopsies were normal and in one case leptomeningeal and cerebral carcinomatous was present.

In the present material 78 of 180 were of value since they fulfilled the abovementioned criteria.

*B. I. Iivemark*

## PROCEEDINGS OF PAEDIATRIC SOCIETIES

### THE FINNISH PAEDIATRIC SOCIETY

Meeting May 27 1969

Tadeusz Chrapowicki (Warsaw Poland) *Polish mother and child welfare*

The population of Poland is c 33.5 million. One half lives in rural areas and  $\frac{1}{3}$  is children.

There were 522 000 live births during last year. The paediatric welfare work takes care of children up to 18 years.

The total number of physicians exceeds 40 000, that of paediatricians 5000 and that of gynecologists and obstetricians 3000. There are 14 paediatricians per 100 000 inhabitants.

There is a continuously increasing number of paediatricians working in narrow subspecialties: paediatric surgery, cardiology, contagious disease, Tb, laryngology, endocrinology, psychiatry, nephrology, oncology etc.

The total number of children's hospital beds is 32 806, that of newborn's beds in special wards 13 500. The number of gynecologic and obstetric beds is 23 000.

Actually 77 per cent of the deliveries take place in hospitals, 18 per cent in rural labour

rooms and 5 per cent at home, under the care of a specialist doctor and midwife.

Family planning is encouraged by the government. Prophylactic examinations of women for cancer have been made in all provinces.

Parallelly to the development of the hospital care, the ambulatory care extends its activity both in general practice and in different specialties. In the central towns of the medical districts more than 20 different specialities are covered.

We pay special attention to the development of sanatoria for the treatment of chronic diseases in children. The number of beds in sanatoria is 8000. Children's mortality has decreased to 33.4 per 1000 live births.

The number of patients with tuberculosis permanently diminishing. In 1969 20 new cases were registered per 100 000 children below 14 years of age.

Two thirds of the schools have own physicians and 1 200 000 school children were sent to summer camps.

Meeting May 30-31 1969

(together with the Polish Paediatric Association)

### SYMPOSIUM OF THE NEWBORN

Irena Żywicka-Twarowska (Poznan Poland) *Electroencephalogram of the asphyctic newborn*

A group of 122 newborn infants asphyctic at birth and expressing symptoms indicating brain

damage were studied together with a control group of 127 healthy newborns. The results of EEG investigations recorded 2 to 11 times in every newborn were correlated to etiological factor of neonatal asphyxia, clinical and neurological picture of the newborn, additional ex-

amniotons gross anatomical and histopathological findings at necropsy and follow up of the infant's development.

Ninety six of 122 newborn babies survived and about half of them showed disturbances in their psychomotor activity. The author demonstrates various types of pathological EEG tracings during the first days of life (paroxysmal features, slow activity, poor activity, marked focal abnormalities, abnormal sleep pattern etc.) and discusses their causes and electrogenesis. EEG investigations during the neonatal period are of importance with respect to: 1) early diagnosis of cerebral damage, 2) prognosis concerning the newborn's life and sometimes later development. Their prognostic value is dependent on persisting abnormal tracings. The best diagnostic and prognostic information is provided by EEG recording started during the first two days of life.

#### Discussion

*A. Hoffman:* Do you always find a normal EEG in normal babies?

*I. Taworniska:* The EEG of the newborn is showing great variations depending on the state of wakefulness, light sleep or deep sleep, but paroxysmal features never occur in healthy newborns.

*Zofia Szczepanska (Poznan, Poland):* Acid base balance during exchange transfusion in newborn infants.

Acid base balance was assessed in 18 newborn infants to whom 24 exchange transfusions were performed because of hemolytic disease and hyperbilirubinaemia of prematurity. The series was divided into two groups according to clinical condition. The first group consisted of 9 newborns with moderate signs of hemolytic diseases but good general condition. In the second group there were 9 infants with severe hemolytic disease and poor general condition at birth and at the beginning of the transfusion. Fifty seven vials of citrate blood used for

transfusion were also checked, the mean pH being 6.50.

In the first group disturbances in acid base balance were found in 3 cases prior to, in 6 cases during and in 6 cases after the transfusion, whereas in the second group disturbed acid base balance was found in nearly all cases. Respiratory and metabolic acidosis was found in infants of the second group prior to the transfusion. Variations of acid base balance during transfusion and a distinct tendency towards alkalosis after transfusion were found in almost all newborns.

*D. Lomaska, J. Herbst, J. Gurdz & M. Soszalskowska (Warsaw, Poland):* Estimation of various chemical and serological factors in the amniotic fluid: its relationship to hemolytic disease of the newborn.

Various examinations of the amniotic fluid were compared to evaluate their suitability for assessing the degree of foetal affection due to blood group incompatibility.

Forty nine specimens of amniotic fluid from 36 Rh immunized cases were examined. The series was divided into 3 groups according to optical density of the amniotic fluid.

The general condition of the newborns corresponded well to the optical density of the amniotic fluid. The mean bilirubin concentrations of the two groups were significantly different. A correlation between bilirubin concentration and optical density could not be confirmed.

Anti D antibodies occurred in some cases in all groups irrespective of the level of antibodies in the mother's blood.

The acid base balance of the amniotic fluid was normal in all groups.

It was concluded that the optical density is the only one of all our examinations of the amniotic fluid which provides useful evaluation of the affection of the foetus in a pregnancy complicated with Rh immunization.



T O A Pääviläinen (Turku Finland) *Foetal tracheobronchial fluid*

The chemical composition of the tracheo-bronchial fluid is different from that of the amniotic fluid. In the foetal lamb it resembles chemically more the plasma than the amniotic fluid.

In our studies sheep and guinea pigs were used as experimental animals. The fetuses were delivered by Caesarean section: sheep under local and guinea pigs under barbiturate anaesthesia. The lowest glucose concentration in both species was observed in the tracheobronchial fluid. In the amniotic fluid of the sheep the glucose concentration was more than four to five times as high as in blood samples. When the glucose level of the maternal blood in the guinea pigs was changed by intravenous glucose infusion, insulin and glucagon, the effect on the glucose concentration of the tracheobronchial fluid of the foetus was very slight during an observation time of one to one and a half hours. Significant differences in cholesterol and creatinine concentrations in the foetal and maternal blood, amniotic fluid and tracheobronchial fluid were found in both species (Pääviläinen *et al.* 1967).

Varying directions of the concentration differences of several substances indicate that the formation of the tracheobronchial fluid cannot be explained as a result of one factor only. Too little is known of the foetal pulmonary transcapillary passage to explain the possible transport of water and other substances between blood and peripheral airways.

## Reference

- Pääviläinen T O A, Hirvonen L & Peltinen T. Glucose, cholesterol and creatinine in the foetal tracheobronchial fluid of the guinea pig and the sheep. *Acta Paediat Scand* 56: 660, 1967.

Krzyszna Buchcar-Huton (Warsaw, Poland) *The role of separate neonatal hospital units in the decrease of mortality*

The Warsaw medical district accounts for nearly 1/11 of the Polish territory with a po-

pulation of c. 2.5 million; the mean number of live births per year was 41 860 in 1964-1968.

In 1960-1964 infant mortality varied from mortality; there was a particularly serious situation at that time, i.e. a considerable number of cases of perinatal trauma and of various diseases.

In 1964 an obstetrical gynaecological department was opened at the Warsaw medical district central hospital with separate beds for cases of abnormal pregnancy. There were 50 beds for newborn and low weight infants and 30 unspecialized district hospitals. The activities of the above mentioned departments in the field of prophylaxis and treatment, intense care and training of the hospital staff, resulted in a significant decrease in infant mortality from 54 ‰ to 38.0. The perinatal mortality decreased from 38.0 to 28.5 and the postnatal mortality from 20.4 to 15.7.

Eero Viiparvuori (Helsinki, Finland) *Respirator treatment in respiratory failure of the newborn* (To be published in *Acta Paediat Scand*)

Tom Sederholm (Helsinki, Finland) *Law prognosis of foetal erythroblastosis*

Although the cytotoxic effect of bilirubin is confirmed, it has never been proved that bilirubin is the only cause of kernicterus. The possible occurrence of less severe cerebral injury following erythroblastosis has not been extensively investigated.

A retrospective study of children treated because of foetal erythroblastosis at the Children's Hospital, University of Helsinki in 1963-1964 was started. Of the 301 children who survived the neonatal period, 92 per cent could be traced by the time of this preliminary report. One child was excluded because of Down's syndrome.

The parents of all children were requested to fill in a questionnaire about the development of their child and the possible occurrence of motor, hearing, speech, psychological and other disturbances.

The mean age at learning to walk was 12.7 months which is about 1 month later than the average for Finnish children. Twenty seven per cent learned to walk at the age of 14 months or later. The mean age at learning to talk sentences was 19.9 months and 25 per cent spoke their first clear sentences at the age of 21 months or later. At the age of 4-5 years one child suffered from athetosis and three from spastic hemiparesis. In addition 17 per cent were thought to be clumsy. Speech disturbances occurred in 40 per cent, 28 per cent tired easily when playing and 11 per cent had a short attention span. Eight per cent of the children were described as hyperactive. Labiality, breath holding spells and antisocial behaviour occurred in 4 per cent. Four per cent of the children had enuresis and 0.4 per cent had encopresis.

Fifty four children with a low cord hemoglobin level were compared to 53 children with a high value and a clear difference in the development was found even when these main groups were divided into sub-groups on the basis of the maximal bilirubin values recorded during the neonatal period. In the group with cord hemoglobin below 10 g per 100 ml and maximum bilirubin over 20 mg per 100 ml the mean age at learning to walk was 14.0 months while the group with low hemoglobin and bilirubin below 20 mg per 100 ml walked at the mean age of 13.1 months. Children with hemoglobin above 15 g per 100 ml and maximal bilirubin over 20 mg per 100 ml walked at the mean age of 11.9 months while those with bilirubin below 20 mg per 100 ml walked at the mean age of 12.4 months. The mean ages at the time of the first clear sentences in these groups were 23.0, 21.2, 20.3 and 19.5 months respectively.

The results seem to indicate that maximal brain dysfunction frequently develops after foetal erythroblastosis and this dysfunction is related both to cord hemoglobin and to the maximal bilirubin level.

In a closer follow up study the children with cord hemoglobin below 10 g per 100 ml were

compared with those with hemoglobin over 15 g per 100 ml. The neurological part of this investigation included a thorough neurological examination, motor development tests according to Oszeretky and EEG. The psychological examination included intelligence tests as well as perceptual function and visual motor co-ordination tests. A Rorschach test was done and phoniatric, otoneurological and ophthalmological examinations were also performed.

So far 47 children have been examined. The preliminary results clearly indicate that although the classical kernicterus syndrome is prevented by the treatment based on the criteria for exchange transfusions presently generally accepted this treatment is not sufficient to prevent the occurrence of slight disturbances such as tremor, ataxia, hyperreflexia, over all clumsiness and co-ordination disturbances. Crossed laterality of the dominant hand and foot occurred as did disturbances in perceptual functions and visual motor co-ordination. The EEG showed frequently slight changes in the background activity but also in some cases hyper synchronous discharges. Slight disturbances in vestibular function and dysfunction of eye movements were common in these children. Disturbances of phonation such as hyperkinesia, dysarthria and dyslalia occurred in a very high percentage of the children.

It was observed that the lower the cord hemoglobin was the more frequent were the manifestations of maximal brain dysfunction regardless of the maximal bilirubin value recorded in the neonatal period.

Clear evidence was presented that the observed abnormalities might be prevented by immediate exchange transfusions performed within the first hours of life.

Irena Zywicka-Twarowska & Jerzyłowska  
Zofia Szczepanińska & A. Brębotowicz (Poznań  
Poland) *Late prognosis on the pathologic con-  
dition of the newborn*

The purpose of this study was the evaluation of factors permanently damaging the newborn.

organism. The subject of investigation were 552 children under observation from the neonatal period up to 4-7 years of age. With respect to the young age this is only a preliminary report.

I. In the group of 75 infants with severe haemolytic disease abnormal development was found in 13.3 per cent. The observations showed that the association of even severe hyperbilirubinemia reaching the level of 25-30 mg per 100 ml with permanent damage of the child was not as apparent as expected. In addition to hyperbilirubinemia other factors such as anoxia, intracranial haemorrhage, hypoglycemia, immaturity, dystrophy, infections and disturbed adaptation may cause a permanent damage of the central nervous system (CNS).

II. In the group of 115 infants delivered by toxemic mothers, abnormal development was found in 9.6 per cent. Permanent lesions in this group of infants could not be proved to result from toxemia of pregnancy, probably because the mothers were treated long time before delivery. There was a probable correlation between disturbed development of the child and intrauterine dystrophy of the foetus. In the neonatal period there was an evident relation between chronic toxemia of pregnancy and intrauterine dystrophy of the newborn. Thus intrauterine dystrophy was observed in 60.5 per cent of pregnancies complicated by chronic and in 13.5 per cent of those with acute toxemia. Additional analysis of smears for date newborn babies revealed that toxemia of pregnancy was the cause of dystrophy only in 28.6 per cent.

III. In the group of 122 infants born in a severe state of asphyxia with Apgar score 0 to 2 points, abnormal development was found in 21.3 per cent. The following obstetric factors bear a high risk for the baby: foetal anoxia in cases of placenta previa or prolapsed cord, infections and abnormal foetal position and labour mechanism. In this group the worst prognosis concerning the later development is occurring with postnatal apnoea lasting for

more than 5 minutes or the presence of severe neurological symptoms persisting over 7 days of life.

IV. In the group of 140 infants with severe damage of the CNS in the neonatal period late abnormal development was found in 61.4 per cent. Specific clinical symptoms persisting for more than 7 days of life appear to be of bad prognostic significance. In most cases the first breath was not delayed. In 10 per cent of the cases coexistence of a congenital malformation of the CNS was found.

In the control group of 100 healthy newborn, abnormal development was found in 1 infants.

#### *Ossi Petäry (Helsinki, Finland) Newborn infections*

At the Children's Hospital, University of Helsinki, several hundred exchange transfusions are done annually. These transfusions are performed through an umbilical venous catheter. Since umbilical catheterization is considered to include a risk for infection, all these patients receive antibiotics prophylactically. Bacterial swabs were taken from the umbilicus and bacterial culture of the blood drawn through the catheter at the beginning and after the exchange transfusion to evaluate the justification of this prophylaxis. The results of the swabs and cultures correlate quite well. If the samples are taken during the first day after birth, less than 10 per cent of the umbilical swabs and blood cultures are positive. The percentage increases to approx. 60 on the fourth day of life. Blood cultures taken from a peripheral vein on the third day of life revealed bacterial growth in 10 per cent. In most but not all cases the bacteria were the same as found in the umbilicus.

Generally speaking, staphylococcus aureus has disappeared as a troublemaker in our newborn wards. The gram-negative bacteria *Pseudomonas*, *Klebsiella* and *Coli* have taken their place. Some of the strains possess a transferable resistance against at least ampicillin and

tetracyclin. The clinical importance of this finding is as far very hard to evaluate. The typing of the *Pseudomonas* strains has not revealed any special pathogenic strains. In contrary one gets the impression that even strains belonging to normal bacterial flora are able to cause disease in these small infants.

Irena Zymalka-Twarowska & R. Włodarszak  
(Poznan, Poland) *Newborn infections*

The results of systematic bacteriological studies performed during a period of 9 months on newborn infants and their environment at the Department of Newborn Pathology were presented.

Gram negative bacteria were prevalent in the newborns and like *Staphylococcus aureus* they bore an extremely high risk for the infant. The incidence of *E. coli* was of an endemic character and various strains were found. During a period of rapid rise in incidence of these bacteria resembling an epidemic haemolytic-producing strains were predominant and a carrier was found among the staff. At the beginning of the study the incidence of *Pseudomonas aeruginosa* strains was very high. Many bacteriological and epidemiological investigations were undertaken in order to find the sources of dissemination (mothers, newborn carrier, water tanks in the incubators, wash bowl outlets) and links in the epidemiological chain (rubber nipples, catheters, suckers). The elimination of the sources and links resulted in the elimination of the *Pseudomonas aeruginosa* from the environment. Examination of the environment showed Gram negative bacteria cultured from the objects which constituted the links in the epidemiological chain in the neonatal wards. The staff was not the source of the Gram negative bacteria.

The incidence of coagulase positive strains of *Staphylococcus aureus* was of endemic character. During a period of rapid rise in incidence epidemiological investigations showed

coagulase positive strains of *Staphylococcus aureus* in several series of powder milk.

Regular co-operation of the clinicians and bacteriologists is essential in preventing neonatal acquired infections and leads to early recognition of epidemics, detection and elimination of sources and links of infection and to general improvement of the organisation of the Newborn Department. Infections of intra-uterine origin constitute the fundamental problem in infections during the neonatal period.

Jukka Valmaki (Turku, Finland) *Neonatal ECG heart rate and respiration patterns in long term recordings* (To be published in *Acta Paediat Scand*)

Antti Korpikallio (Turku, Finland) *Effects of metabolic acidosis on the circulation of newborns* (The presented paper represents a part of a more extensive study. Cardiovascular response to hypoxia, hypercapnia and metabolic acidosis. *Acta Paediat Scand Suppl* 191 1969)

Santo Javkka (Turku, Finland) *Skin temperature variation and acid base balance during the first minutes of life*

The purpose of the present work was to determine the normal skin temperature variation and the acid base balance in the first minutes of life.

The series consisted of 76 newborn infants. The skin temperatures of all the newborn were recorded and simultaneously thermocouples were taken. From each infant a sample of cord blood was taken immediately after birth.

The normal skin temperature curve is a typical one. An initial rapid rise is followed by a drop in temperature. The first sign of a rise after this drop occurs within 10 min after birth. The mean time up to the permanent rise was 6.9 min and the mean blood pH was 7.29. In acidotic newborn the rise in temperature was slightly delayed. However the delayed term

organism. The subject of investigation were 552 children under observation from the neonatal period up to 4-7 years of age. With respect to the young age this is only a preliminary report.

I. In the group of 75 infants with severe haemolytic disease abnormal development was found in 13.3 per cent. The observations showed that the association of even severe hyperbilirubinaemia reaching the level of 25-30 mg per 100 ml with permanent damage of the child was not as apparent as expected. In addition to hyperbilirubinaemia, other factors such as anoxia, intracranial haemorrhage, hypoglycaemia, immaturity, dystrophy, infections and disturbed adaptation may cause a permanent damage of the central nervous system (CNS).

II. In the group of 115 infants delivered by toxæmic mothers abnormal development was found in 9.6 per cent. Permanent lesions in this group of infants could not be proved to result from toxæmia of pregnancy probably because the mothers were treated long time before delivery. There was a probable correlation between disturbed development of the child and intrauterine dystrophy of the foetus. In the neonatal period there was an evident relation between chronic toxæmia of pregnancy and intrauterine dystrophy of the newborn. Thus intrauterine dystrophy was observed in 60.5 per cent of pregnancies complicated by chronic and in 13.5 per cent of those with acute toxæmia. Additional analysis of small for date newborn babies revealed that toxæmia of pregnancy was the cause of dystrophy only in 28.6 per cent.

III. In the group of 122 infants born in a severe state of asphyxia with Apgar score 0 to 2 points abnormal development was found in 21.3 per cent. The following obstetric factors bear a high risk for the baby: foetal anoxia, in cases of placenta previa or prolapsed cord, infections and abnormal foetal position and labour mechanism. In this group the worst prognosis concerning the later development is occurring with postnatal apnoea lasting for

more than 5 minutes or the presence of severe neurological symptoms persisting over 7 days of life.

IV. In the group of 140 infants with severe damage of the CNS in the neonatal period later abnormal development was found in 61.4 per cent. Specific clinical symptoms, persisting for more than 7 days of life appear to be of bad prognostic significance. In most cases the first breath was not delayed. In 10 per cent of the cases coexistence of a congenital malformation of the CNS was found.

In the control group of 100 healthy newborn abnormal development was found in 2 infants.

#### Ossi Pettay (Helsinki, Finland) *Newborn infections*

At the Children's Hospital, University of Helsinki, several hundred exchange transfusions are done annually. These transfusions are performed through an umbilical venous catheter. Since umbilical catheterization is considered to include a risk for infection, all these patients receive antibiotics prophylactically. Bacterial swabs were taken from the umbilicus and bacterial culture of the blood drawn through the catheter at the beginning and after the exchange transfusion to evaluate the justification of this prophylaxis. The results of the swabs and cultures correlate quite well. If the samples are taken during the first day after birth, less than 10 per cent of the umbilical swabs and blood cultures are positive. The percentage increases to approx. 60 on the fourth day of life. Blood cultures taken from a peripheral vein on the third day of life revealed bacterial growth in 10 per cent. In most but not all cases the bacteria were the same as found in the umbilicus.

Generally speaking, *Staphylococcus aureus* has disappeared as a troublemaker in our newborn wards. The gram-negative bacteria *Pseudomonas*, *Klebsiella* and *Coli* have taken their place. Some of the strains possess a transferable resistance against at least ampicillin and

tetracyclin. The clinical importance of this finding is so far very hard to evaluate. The typing of the *Pseudomonas* strains has not revealed any special pathogenic strains. In contrast one gets the impression that even strains belonging to normal bacterial flora are able to cause disease in these small infants.

Irena Zywiła-Turkowiak & K. Włodarczyk  
(Poznań, Poland) *Newborn infections*

The results of systematic bacteriological studies performed during a period of 9 months on newborn infants and their environment at the Department of Newborn Pathology were presented.

Gram-negative bacteria were prevalent in the newborns and like *Staphylococcus aureus* they bore an extremely high risk for the infant. The incidence of *E. coli* was of an endemic character and various strains were found. During a period of rapid rise in incidence of these bacteria resembling an epidemic haemolysing strains were predominant and a carrier was found among the staff. At the beginning of the study the incidence of *Pseudomonas aeruginosa* strains was very high. Many bacteriological and epidemiological investigations were undertaken in order to find the sources of dissemination (mothers' newborn carriers, water tanks in the incubators, wash bowl outlets) and links in the epidemiological chain (rubber nipples, catheters, suckers). The elimination of the sources and links resulted in the elimination of the *Pseudomonas aeruginosa* from the environment. Examination of the environment showed Gram-negative bacteria cultured from the objects which constituted the links in the epidemiological chain in the neonatal wards. The staff was not the source of the Gram-negative bacteria.

The incidence of coagulase-positive strains of *Staphylococcus aureus* was of endemic character. During a period of rapid rise in incidence epidemiological investigations showed

coagulase-positive strains of *Staphylococcus aureus* in several series of powder milk.

Regular co-operation of the clinicians and bacteriologists is essential in preventing neonatal acquired infections and leads to early recognition of epidemics, detection and elimination of sources and links of infection and to general improvement of the organisation of the Newborn's Department. Infections of intrauterine origin constitute the fundamental problem in infections during the neonatal period.

Ulla Valmaki (Turku, Finland) *Neonatal ECG heart rate and respiration patterns in long term recordings* (To be published in *Acta Paediatr Scand*)

Antti Korvinko (Turku, Finland) *Effects of metabolic acidosis on the circulation of newborns* (The presented paper represents a part of a more extensive study: Cardiovascular response to hypoxia, hypercapnia and metabolic acidosis. *Acta Paediatr Scand* Suppl. 101, 1969).

Santo Jaykka (Turku, Finland) *Skin temperature variation and acid base balance during the first minutes of life*

The purpose of the present work was to determine the normal skin temperature variation and the acid base balance in the first minutes of life.

The series consisted of 76 newborn infants. The skin temperatures of all the newborn were recorded and simultaneously thermocouple pictures were taken. From each infant a sample of cord blood was taken immediately after birth.

The normal skin temperature curve is a typical one. An initial rapid rise is followed by a drop in temperature. The first sign of a rise after this drop occurs within 10 min after birth. The mean time up to the permanent rise was 6.9 min and the mean blood pH was 7.29. In acidotic newborn the rise in temperature was slightly delayed. However the delayed tem-

organism. The subject of investigation were 552 children under observation from the neonatal period up to 4-7 years of age. With respect to the young age this is only a preliminary report.

I. In the group of 75 infants with severe haemolytic disease abnormal development was found in 13.3 per cent. The observations showed that the association of even severe hyperbilirubinaemia reaching the level of 25-30 mg per 100 ml with permanent damage of the child was not as apparent as expected. In addition to hyperbilirubinaemia other factors such as anoxia, intracranial haemorrhage, hypoglycaemia, immaturity, dystrophy, infections and disturbed adaptation may cause a permanent damage of the central nervous system (CNS).

II. In the group of 115 infants delivered by toxemic mothers abnormal development was found in 9.6 per cent. Permanent lesions in this group of infants could not be proved to result from toxemia of pregnancy, probably because the mothers were treated long time before delivery. There was a probable correlation between disturbed development of the child and intrauterine dystrophy of the foetus. In the neonatal period there was an evident relation between chronic toxemia of pregnancy and intrauterine dystrophy of the newborn. Thus intrauterine dystrophy was observed in 60.5 per cent of pregnancies complicated by chronic and in 13.5 per cent of those with acute toxemia. Additional analysis of small for date newborn babies revealed that toxemia of pregnancy was the cause of dystrophy only in 28.6 per cent.

III. In the group of 122 infants born in a severe state of asphyxia with Apgar score 0 to 2 points abnormal development was found in 21.3 per cent. The following obstetric factors bear a high risk for the baby: foetal anoxia in cases of placenta previa or prolapsed cord, infections and abnormal foetal position and labour mechanism. In this group the worst prognosis concerning the later development is occurring with postnatal apnoea lasting for

more than 5 minutes or the presence of severe neurological symptoms persisting over 7 days of life.

IV. In the group of 140 infants with severe damage of the CNS in the neonatal period later abnormal development was found in 61.4 per cent. Specific clinical symptoms persisting for more than 7 days of life appear to be of bad prognostic significance. In most cases the first breath was not delayed. In 10 per cent of the cases coexistence of a congenital malformation of the CNS was found.

In the control group of 100 healthy newborn abnormal development was found in 2 infants.

#### Ossi Pettay (Helsinki, Finland) *Newborn infections*

At the Children's Hospital, University of Helsinki, several hundred exchange transfusions are done annually. These transfusions are performed through an umbilical venous catheter. Since umbilical catheterization is considered to include a risk for infection, all these patients receive antibiotics prophylactically. Bacterial swabs were taken from the umbilicus and bacterial culture of the blood drawn through the catheter at the beginning and after the exchange transfusion to evaluate the justification of this prophylaxis. The results of the swabs and cultures correlate quite well. If the samples are taken during the first day after birth, less than 10 per cent of the umbilical swabs and blood cultures are positive. The percentage increases to approx. 60 on the fourth day of life. Blood cultures taken from a peripheral vein on the third day of life revealed bacterial growth in 10 per cent. In most but not in all cases the bacteria were the same as found in the umbilicus.

Generally speaking, *Staphylococcus aureus* has disappeared as a troublemaker in our newborn wards. The gram-negative bacteria *Pseudomonas*, *Klebsiella* and *Coli* have taken their place. Some of the strains possess a transferable resistance against at least ampicillin and

central regions in all children. It is interesting that it is now appearing centrally before appearing occipitally.

The latent period of the first deflection of the visually evoked potentials is shortened rapidly within the first three months of life. Later on the latency is reduced only slowly until school age when the standard of the adult is reached. The latency of the negative wave of a premature infant was 238.8 msec during the 34th postconceptional week. At term the latency of the primary positive deflection amounted to 218.3 msec and decreased during the first three months after term to 152.1 msec. During school age the latent period was only 118 msec. Thus during the first three months of life the latency is reduced by one third.

The changes of the visually evoked potentials reflect the maturation of the brain in childhood. During the first three to four months of life the decisive maturation of the visual cortex takes place. During this period the typical pattern of the layers of area 17 (area striata) is extending to the top of the gyrus while the myelination is proceeding. At the same time the dendrites are branching out strongly and the axosomatic synapse begins to

predominate over the axodendritic synapses. The morphological changes are associated with changes of the visually evoked potentials which thus reflect the maturation of the brain. At the age of six to seven years the visual cortex is completely matured. This corresponds with the termination of the differentiation of the visually evoked potentials.

The latent period of the visually evoked potentials is dependent on brain maturity and can thus be used as a very reliable method for the estimation of the conceptional age in small for date babies. The accuracy of this measurement corresponds with the accuracy of nerve conduction velocity determination.

In children with progressing internal hydrocephalus already a slight enlargement of the ventricles leads to a deformation of the visually evoked potentials. The most constant change is the marked prolongation of the latency which increases together with the progression of the internal hydrocephalus.

The visually evoked potentials constitute a valuable complement to the diagnostic tools especially to the retinogram when diseases of the visual system are studied.

*E. I. Wallgren*



perature rise was not always a sign of increased acidosis. After complicated pregnancies and deliveries the final balance of thermoregulation was delayed, even though the infant's condition otherwise appeared to be good.

The authors are of the opinion that the variation of the skin temperature of a newborn infant reflects disturbances in the condition of the newborn and that its measurement can be used as a clinical aid in the evaluation of the newborn.

Kalle Österlund, Esko Tahlu & John Lind  
(Helsinki, Finland) *Cooling of the newborn recorded by cinethermography*

The variation of skin temperature in different parts of the newborn infant's body was demonstrated in a film showing cinethermographic recordings during the delivery and the first minutes of life.

#### Meeting June 11 1969

H. Fichsel (Bonn, West Germany) *Visually evoked potentials in cerebral cortex of children. Development of responsiveness and changes under pathological conditions*

Evoked potentials are released in the visual cortex by stimulation of the retina with light flashes. These potentials can be released from the union or the occipital regions in children of every age and it is possible to record them from the scalp.

The visually evoked potentials were studied in 256 premature and full term newborn infants with the aid of a CAT 400. Furthermore 10 infants with internal hydrocephalus were examined.

The progressive development of the visually evoked potentials, beginning with the foetal stage and continuing to the end of school age, is characterized by

1. differentiation of the number, shape and sequence of the waves
2. the topographic spread of the waves
3. shortening of the latent period

Visually evoked potentials can be released in every instance during the second trimester of the foetal period. Up to the eighth postconceptional month the visually evoked potentials consist of a primary negative deflection followed by a positive deflection. Shortly before birth and during the newborn period the typical potentials show a complex of a positive deflection followed by a negative and final posi-

tive deflection. From the third month of life an important differentiation takes place: a negative wave appears in front of this complex showing a rapidly increasing amplitude. The duration of the following large positive deflection increases together with its amplitude. Although the amplitude of the big positive wave then again decreases towards the end of the first year of life, the duration of this deflection increases steadily. At the end of the first year of life another complex consisting of a negative and a positive wave appears, preceding these potentials. This complex represents the first and the second wave of the final evoked potentials. At the beginning of school age the shape and the number of waves of the child's visually evoked potentials have almost attained their adult pattern.

The visually evoked potentials of premature newborn infants have their maximal intensity over the union. They are very distinct over the parasagittal occipital regions and can be demonstrated over parietal regions in 70 per cent of the cases and over central regions in 15 per cent of the cases. In full term newborn infants the maximum is likewise over the union but there is less spreading of the potentials. During the third month of life the maximum of the visually evoked potentials is changing from the union to the occipital regions while there is hardly any spread over the non-visual cortex. After the sixth year of life a negative potential can again be observed as well in parietal as in

## ANNOUNCEMENT

### Heinz Karger Prize 1969

The prize which is awarded every year in memory of Heinz Karger the wellknown Basle publisher for an outstanding scientific work has in 1969 been conferred in equal parts to Edit Beregi Budapest and Alex Comfort London for their papers on the subject of Basic Research in Gerontology

The Heinz Karger Memorial Foundation invites the submission of papers on the following subjects

1970 An original paper on *Computers in Medical Diagnosis*

1971 An original paper on *Biochemical Aspects of Immunology*

#### Conditions

Manuscripts on the subject for 1970 should not exceed 30 typewritten pages and manuscripts on the subject for 1971 should not ex-

ceed 20 typewritten pages including illustrations tables and bibliography Manuscripts marked Competition must reach the publishers S Karger AG Arnold Bocklin Strasse 25 CH-4000 Basle 11 (Switzerland) not later than June 30 The manuscript must be typewritten on one side only double spaced and is to be submitted in duplicate and in accordance with the instructions contained in The Manuscript (Rules for the preparation of manuscripts and bibliographies of scientific papers) This booklet can be obtained free of charge from the publishers if the request is marked Competition

Language English German or French

Publication The winning papers will be published in one of the Karger journals

The award for the prize will be SFr 5000 - The Council of the Foundation will judge the papers and confer the prizes

## NEW BOOKS RECEIVED

- V Dubowitz *The floppy infant* Clinics in Developmental Medicine No 31 109 pp W Heinemann Medical Books Ltd London 1969 US \$6.00
- D G Evans (ed) *Immunity against infectious diseases* British Medical Bulletin Vol 25 No 2 London 1969 £2
- H J Krüfmann (ed) *Progress in pediatric radioLOGY* Vol 2 *Gastrointestinal tract* 403 pp illus S Karger AG Basel New York 1969 DM 85.— 170s
- A Benedikt & P Csáki *Zur Frage der Entwicklung von Kindern mit niedrigem Geburtsgewicht* 167 pp Akadémiai Kiadó Budapest 1969 US \$6.00
- C C de Silva & N G Birtvist *Tropical nutritional disorders of infants and children* 226 pp Charles C Thomas Publ Springfield Ill 1969 US \$11.50
- U Willenbockel *Zur Physiologie und Pathologie des Phosphatstoffwechsels* 109 pp illus Ferdinand Enke Verlag Stuttgart 1969 DM 30.—
- J G Howells (ed) *Modern perspectives in international child psychiatry* 878 pp Oliver & Boyd Ltd Edinburgh 1969 £8.8s
- W Zublin *Chromosomale Aberrationen und Psyche* 160 pp Bibliotheca Psychiatrica et Neurologica No 140 S Karger AG Basel New York 1969 US \$10.10
- H L Barnett (ed) *Pediatrics—14th Edn* 1847 pp illus Butterworth & Co Ltd London 1969 £10.15s
- P G Kiss *Über die Grundlagen der klinischen Psychologie* 273 pp Akadémiai Kiadó Budapest 1968 US \$9.60
- J Brodehl *Der renale Transport der Ammoniumionen im Säuglings- und Kindesalter* 97 pp illus Beihfte z Archiv f Kinderheilkunde 61 Heft Ferdinand Enke Verlag Stuttgart 1969 DM 30.—
- H Opitz & F Schmid (eds) *Handbuch der Kinderheilkunde* Band VIII/1 *Neurologie Psychologie Psychiatrie* F Schmid & H Asperger eds 1060 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 385.—
- G Myse *Croissance et développement de l'enfant à Dakar* 263 pp Centre International de L'Enfance Paris 1969 Price not given
- The Cardiff Diagnostic Classification* 163 pp British Paediatric Association London 1969
- M M Ravitch *Repair of hernias* 189 pp illus Year Book Medical Publ Inc Chicago Ill 1969 US \$10.00

## PLASMA HEMOPEXIN AND HAPTOGLOBIN IN HEMOLYTIC DISEASES OF THE NEWBORN

BENGT LUNDH, FRANK A. OSKI and FRANK H. GARDNER

From the Hematology Research Laboratory, Presbyterian University of Pennsylvania Medical Center, Department of Pediatrics, Hospital of the University of Pennsylvania and the Departments of Medicine and Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Many factors have been considered in the evaluation of hemolytic diseases of the newborn. At the present time it may be difficult to estimate the degree of hemolysis accurately, the risks for exchange transfusion and the effect of such a transfusion. Recently it has been shown that the concentration of hemopectin, a glycoprotein with high affinity for heme, is increased in hemolytic diseases in adults and older children (10, 15). Also the hemopectin level in the normal newborn infant is about 20% of the normal adult level (3). In the present study the plasma hemopectin concentration in a group of normal term and preterm infants as well as newborn infants with hemolytic diseases has been determined with an immunodiffusion method in an attempt to assess its value as an aid in the diagnosis of increased red cell destruction. Although it is well known that the haptoglobin level in newborn infants is generally very low (2, 12, 14), immunochemical determinations of haptoglobin were included to evaluate the relationship between these two plasma proteins in the neonatal period.

### METHODS

Blood was taken by heel stab or venopuncture into a heparinized capillary tube. The red cells were separated by centrifugation.

The antisera were kindly supplied by professor C. B. Laurell, Dept of Clin Chemistry, Malmo General Hospital, Malmo, Sweden.

Immediately centrifuged, the plasma portion was separated, sealed and frozen at  $-20^{\circ}\text{C}$ . For the determination of hemopectin and haptoglobin the electroimmunodiffusion method of Laurell was used with specific antisera for hemopectin and haptoglobin. The concentration of antiserum was 0.35 and 0.10 ml/100 ml respectively and 4.5  $\mu\text{l}$  of plasma diluted 1:5 with physiological saline was applied to the plate. The separation time was 4 hours. The height of the peaks was read with the aid of a magnifying lens graded in 0.1 mm. To obtain a standard curve a serum pool from 15 healthy male blood donors was used. A daily sample of the pool was thawed and diluted 1:10, 1:20, 1:40 and 1:80 with saline. These dilutions were then applied in duplicate on every plate. The serum pool was calibrated with the aid of a standardized human serum (Behringwerke) for hemopectin and by the method of Tarukoski for haptoglobin. The concentration of hemopectin in the pool was found to be 63 mg/100 ml and that of haptoglobin 67 mg/100 ml given as hemopectin binding capacity (HbBC). In the following the haptoglobin results are given as HbBC. All determinations were made in duplicate. The variation coefficient of this method was calculated on the basis of 22 double determinations performed on the same plate. For hemopectin it was found to be 6% of a mean concentration of 26 mg/100 ml and for haptoglobin 9% of a mean concentration of 21 mg/100 ml. In a material of 12 samples with a mean hemopectin and haptoglobin concentration of 4.9 and 8.2 mg/100 ml respectively the variation coefficient of the method was found to be 8% for the hemopectin and 7% for the haptoglobin method.

With the methods used a comparison was made between hemopectin and haptoglobin levels in heparinized plasma and serum respectively in varying dilutions from two healthy adults. No significant differences could be demonstrated. The methods were tested on a group of 24 healthy male blood donors aged 18-25 years. The mean hemopectin level was

## ACKNOWLEDGEMENT

The Editorial Board of *Acta Paediatrica Scandinavica* wishes to express its sincere gratitude to the following persons outside the Advisory Board who have acted as referees during the past year. The standard of the journal depends to a very large extent on the skill and interest of these reviewers.

E. A. Ahvenainen

Nils Almqvist

Hugo Andersson

Anita Aperia

Gordon C. Arneil

Gunilla Berglund

R. Broderson

G. Buccini

Lars Erik Carlgren

Methven Cathro

Olof Celander

Arne Dahlqvist

Hans Ekblad

Hans Ericsson

Nils Olof Ericsson

Max Friedman

Ingrid Gimsborg

John Gentz

Lars Ake Hanson

Bertil Hall

Anders Hulth

Bjorn Iversen

Torben Iversen

Andreas Killander

C. O. Kindmark

Bengt Kjellman

Lennart Kohler

Ingemar Ljergard

Bernhard Lindtman

Carl Bertil Lurell

Tor Lindberg

Bo Lindblad

Folke Linell

Sven Erik Lindell

Jan Lindsten

Karl Martin Lundmark

Bertil Magnusson

Gunnar Meeuwisse

Johannes Melchior

Magnus Michrén

Sven Oskar Nettelblad

Ingrid Marie Nilsson

Ingvar Nylander

Patrick Olin

Ingemar Petersen

Bengt Persson

Gustaf Pettersson

Ossi Petty

H. F. R. Prechtl

Ove Qvist

Goran Sterner

Goran Sterky

Leonard Strang

Wilfried v. Studnitz

J. M. Tanner

Walter Teller

Clas Thorén

Ragnar Tunell

Goran Wallgren

Zoe Walsch

Jan Winberg

Lars Wranne

Judson J. Van Wyk

Per Arne Ockerman

Jacob Öster

## PLASMA HEMOPEXIN AND HAFTOGLOBIN IN HEMOLYTIC DISEASES OF THE NEWBORN

BENGT LUNDH, FRANK A. OSKI and FRANK H. GARDNER

*From the Hematology Research Laboratory, Presbyterian University of Pennsylvania Medical Center, Department of Pediatrics, Hospital of the University of Pennsylvania and the Departments of Medicine and Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.*

ry factors have been considered in the evaluation of hemolytic diseases of the newborn. At the present time it may be difficult to estimate the degree of hemolysis accurately, the effects on exchange transfusion and the effect of such a transfusion. Recently it has been shown that the concentration of hemopectin, a glycoprotein with high affinity for heme, is raised in hemolytic diseases in adults and older children (10-15). Also the hemopectin level in the normal newborn infant is about 20% of the normal adult level (3). In the present study the plasma hemopectin concentration in a group of normal term and premature infants as well as newborn infants with hemolytic diseases has been determined with an immunodiffusion method in an attempt to assess its value as an aid in the diagnosis of increased red cell destruction. Although it is well known that the haptoglobin level in newborn infants is generally very low (2, 12, 14), immunochromatological determinations of haptoglobin were included to evaluate the relationship between these two plasma proteins in the neonatal period.

### METHODS

Blood was taken by heel stab or venipuncture into a separated capillary tube. The red cells were im-

The sera were kindly supplied by professor C. B. Laurell, Dept. of Clin. Chemistry, Malmo General Hospital, Malmo, Sweden.

mediately centrifuged, the plasma portion was separated, sealed and frozen at  $-20^{\circ}\text{C}$ . For the determination of hemopectin and haptoglobin the electroimmunodiffusion method of Laurell was used with specific antisera for hemopectin and haptoglobin. The concentration of antisera was 0.35 and 0.10 ml/100 ml respectively and 4.5  $\mu\text{l}$  of plasma diluted 1:5 with physiological saline was applied to the plate. The separation time was 4 hours. The height of the peaks was read with the aid of a magnifying lens graded in 0.1 mm. To obtain a standard curve a serum pool from 15 healthy male blood donors was used. A daily sample of the pool was thawed and diluted 1:10, 1:20, 1:40 and 1:80 with saline. These dilutions were then applied in duplicate on every plate. This serum pool was calibrated with the aid of a standardized human serum (Behringwerke) for hemopectin and by the method of Tarvitski for haptoglobin. The concentration of hemopectin in the pool was found to be 63 mg/100 ml and that of haptoglobin 67 mg/100 ml given as hemopectin binding capacity (HbPC). In the following the haptoglobin results are given as HbPC. All determinations were made in duplicate. The variation coefficient of the method was calculated on the basis of 22 double determinations performed on the same plate. For hemopectin it was found to be 6% of a mean concentration of 26 mg/100 ml and for haptoglobin 9% of a mean concentration of 1 mg/100 ml. In a material of 12 samples with a mean hemopectin and haptoglobin concentration of 4.9 and 8.2 mg/100 ml respectively the variation coefficient of the method was found to be 8% for the hemopectin and 7% for the haptoglobin method.

With the methods used a comparison was made between hemopectin and haptoglobin levels in heparinized plasma and serum respectively in varying dilutions from two healthy adults. No significant differences could be demonstrated. The methods were tested on a group of 24 healthy male blood donors aged 18-55 years. The mean hemopectin level was

# ACKNOWLEDGEMENT

The Editorial Board of *Acta Paediatrica Scandinavica* wishes to express its sincere gratitude to the following persons outside the Advisory Board who have acted as referees during the

past year. The standard of the journal depends to a very large extent on the skill and interest of these reviewers.

E. K. Ahvenainen  
Nils Alwall  
Hugo Andersson  
Anita Aperia  
Gavin C. Arneil  
Gunilla Berglund  
R. Brodersen  
G. Bucci  
Lars Erik Carlgren  
Methven Cathro  
Olof Celander  
Arne Dahlqvist  
Hans Ekelund  
Hans Ericsson  
Nils Olof Ericsson  
Max Friedman  
Ingrid Grunstorp  
Johan Gentz  
Lars Åke Håkanson  
Bertil Hall  
Anders Hulth  
Bjorn Ivarmark  
Torben Iversen  
Andreas Kallander  
C. O. Kindmark  
Bengt Kjellman  
Lennart Kohler  
Ingemar Lagergård  
Bernhard Landtman  
Cari Bertil Laurell  
Tor Lindberg  
Bo Lindblad  
Folke Linell

Sven Erik Lindell  
Jan Lindsten  
Karl Martin Lundmark  
Bertil Magnusson  
Gunnar Meeuwisse  
Johannes Melchior  
Magnus Michaëlsson  
Sven Caspar Nettelblad  
Inga Marie Nilsson  
Ingvar Nylander  
Patrick Olin  
Ingemar Petersen  
Bengt Persson  
Gustaf Pettersson  
Ossi Pettay  
H. F. R. Precht  
Ove Qvist  
Goran Sterner  
Goran Sterky  
Leonard Strang  
Wilfried v. Studnitz  
J. M. Tanner  
Walter Teller  
Clas Thoren  
Ragnar Tunell  
Goran Wallgren  
Zoe Walsch  
Jan Winberg  
Lars Wranne  
Judson J. Van Wyk  
Per Arne Öckerman  
Jacob Øster

a statistically probably significant ( $0.05 > p > 0.01$ )

Fifteen of the 39 term infants had no demonstrable haptoglobin while in the remainder of the group values as high as 70 mg/100 ml were seen. The mean concentration was 13 mg/100 ml (medium 12 mg/100 ml) and the standard deviation 17.4 mg/100 ml. Among the 27 premature infants 19 had no demonstrable haptoglobin while among the others values up to 26 mg/100 ml were observed. The mean was 3 mg/100 ml the medium 0 mg/100 ml and the standard deviation 6.1 mg/100 ml.

The material of term infants was further more divided into two groups one consisting of cases with demonstrable haptoglobin in plasma and the other with no haptoglobin in plasma. The hemoexin level in the first group was  $23 \pm 7.8$  mg/100 ml (mean  $\pm 1$  s.d.) and in the second group  $17 \pm 8.3$  mg/100 ml. The difference was probably significant ( $0.05 > p > 0.01$ ).

Of eight infants in group A one of which was premature with frank hemolysis explained by either a positive Coombs test or G6PD deficiency the hemoexin level was low and varied between 4 and 16 mg/100 ml. Six of these children had no haptoglobin demonstrable while two had haptoglobin levels of 7 and 12 mg/100 ml respectively (Fig. 2). In group B with 4 term and one premature infant with weakly positive Coombs test or G6PD deficiency but without signs of frank hemolysis the hemoexin level was normal and varied between 4 and 28 mg/100 ml with the one low value seen in the premature infant. Haptoglobin could be demonstrated in all these children in concentrations varying between 4 and 25 mg/100 ml (Fig. 2). In group C composed of infants with G6PD deficiency and bilirubin above 8 mg/100 ml but without reticulocytosis or other signs of hemolysis the hemoexin levels were within the normal range and in two of the three cases haptoglobin could be found in relatively high concentration (Fig. 2).

Of the four patients studied before and for varying periods after exchange transfusions

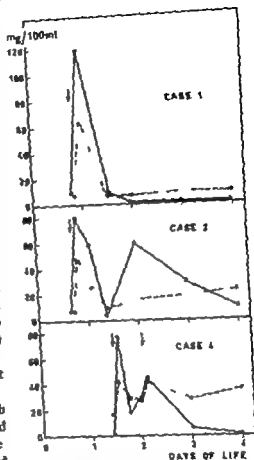


Fig. 3. Serial determinations of hemoexin (---) and haptoglobin (—) in three infants (cases 1, 2 and 4) treated with exchange transfusion (†) due to maternal isosensitization. The transfusions are indicated by arrows.

due to maternal isosensitization (Fig. 3) three (cases 1, 2 and 3) had an initial hemoexin level of 6 mg/100 ml. Immediately after the exchange transfusion substantial increases varying from 40 to 66 mg/100 ml were observed. Two of the cases thereafter showed a rapid decrease to the pretransfusion level followed by a slow increase during the following days. Case 4 initially had a normal hemoexin level of 16 mg/100 ml with a moderate increase after the transfusion to 43 mg/100 ml and a decrease to 27 mg/100 ml 14 hours later. A second exchange transfusion slightly increased the level to 42 mg/100 ml whereafter the concentration stabilized at approximately 30 mg/100 ml.



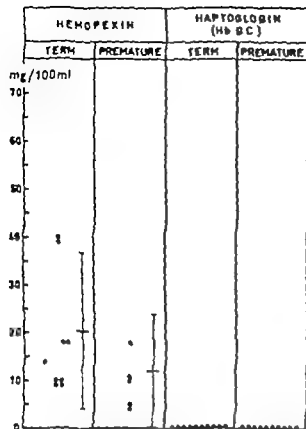


Fig 1 The plasma concentration of hemopexin and haptoglobin in 39 term and 27 premature infants on the first day of life. To the right of the hemopexin results the means  $\pm$  2 SD are indicated

found to be 73 mg/100 ml with a standard deviation of 9 mg/100 ml and the mean haptoglobin level 88 mg/100 ml with a standard deviation of 37 mg/100 ml

## MATERIAL

Plasma was obtained within 24 hours of the time of delivery in all but one instance. Thirty nine of the newborns with a normal hemoglobin hematocrit reticulocyte count blood smear glucose 6 phosphate dehydrogenase (G6PD) activity of the red cells a negative Coombs test and a normal gestational age were considered as normal term infants. A second group comprised 27 hemiotologically normal but premature infants. The gestational age was defined according to the criteria of Koenigsberger and Usher *et al*. All infants less than 38 weeks gestation were classified as premature. The remainder of the material was divided into three groups.

A Term and premature newborns with positive Coombs test (7 patients) or G6PD deficiency (1 patient) and with reticulocytosis  $>6$  and a bilirubin  $>8$  mg/100 ml on the third day of life.

B Term and premature newborns with (usually weakly) positive Coombs test (2 patients) or G6PD deficiency (3 patients) but without signs of reticulo-

hemolysis (reticulocytes below 6 and bilirubin low 8 mg/100 ml on the third day of life).

C Two term and 1 premature newborn. G6PD deficiency and a bilirubin of more than 8 mg/100 ml on the third day of life but without reticulocytosis or other signs of hemolysis.

Finally in four cases included in group A, samples were obtained before and varying periods after exchange transfusions for hyperbilirubemia second to maternal isoimmunization. In one of these the first sample was obtained during the second of life.

## RESULTS

The mean plasma hemopexin level on the first day of life in the 39 term infants was 31 mg/100 ml with a standard deviation of 8.1 mg/100 ml and a range between 8 and 42 mg/100 ml. In the 27 premature infants the mean plasma hemopexin level was 12 mg/100 ml with a standard deviation of 6.3 mg/100 ml and a range between 2 and 26 mg/100 ml (Fig 1). The difference between the mean hemopexin level in term and premature infants

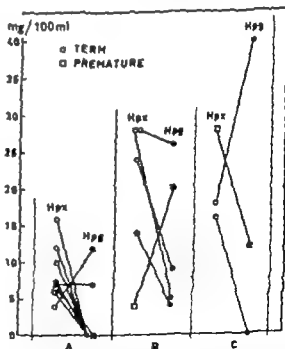


Fig 2 The concentration of hemopexin and haptoglobin in infants with positive Coombs test or G6PD deficiency. The infants in group A had signs of frank hemolysis in group B no signs of hemolysis and in group C hyperbilirubemia only.  $\circ$  Term infants  $\square$  premature infants. Hpx hemopexin Hpg haptoglobin.

high values but reappraisal of their clinical charts did not show any signs of complicating disease such as infection that could be responsible for the high values. The wider fluctuations and the more rapid decrease of the haptoglobin concentration after exchange transfusions may support the hypothesis that haptoglobin acts as the first line of defense in hemolysis and that hemopexin is affected only when the haptoglobin reaches the zero level and the plasma concentration of hemoglobin and of heme increases. It is remarkable that in two of the exchange transfused cases a spontaneous increase in the haptoglobin level was seen after the initial rapid decline. This elevation possibly may be explained by a release from an immature or overloaded reticulo-endothelial system of haptoglobin or haptoglobin split products reacting with the antiserum.

In the group of 8 patients with frank hemolysis due to nonimmunization or G6PD deficiency, low hemopexin levels were seen although 4 cases were within the lower limit of the normal range. Cases with positive Coombs test or with G6PD deficiency but without signs of increased hemolysis all had hemopexin values within the normal range. We have at the moment no explanation for the fact that two of the cases in group A with grossly evident hemolysis and a low hemopexin concentration had significant amounts of haptoglobin in their plasma.

Even if the results of the present study indicate that a hemopexin level below 2 mg/100 ml in a term infant is a sign of pathologically increased red cell turn over the overlap between the groups of term infants with and without grossly evident hemolysis is big. Since this overlap is even bigger among the premature infants due to their lower normal hemopexin level the conclusion must be drawn that there is little value in a hemopexin determination to evaluate the degree of hemolysis in the newborn.

### SUMMARY

Using electromunodiffusion methods the concentrations of hemopexin and haptoglobin

in plasma were determined in 39 newborn term infants, 27 premature infants and 16 infants with G6PD deficiency or positive Coombs test with and without signs of frank hemolysis. The hemopexin concentration in term infants was found to be  $21 \pm 8.1$  mg/100 ml (mean  $\pm$  1 s.d.) and in premature infants  $12 \pm 6.3$  mg/100 ml. The mean haptoglobin concentration for the term infants was 13 mg/100 ml with a range of 0-70 mg/100 ml and for the premature infants 3 mg/100 ml with a range of 0-26 mg/100 ml expressed as hemoglobin binding capacity (HbBC). Only 4 out of 8 infants with positive Coombs test or G6PD deficiency and with signs of frank hemolysis had a significantly decreased hemopexin level. In 6 of these cases no haptoglobin was demonstrable. Infants with G6PD deficiency or positive Coombs test but without signs of hemolysis had a normal hemopexin level and haptoglobin could be found in all of these samples. Immediately after exchange transfusions normal or high hemopexin values were seen with a rapid decline usually within 12 hours to subnormal values. It is concluded that due to the overlap in hemopexin concentration between children with and without grossly evident hemolysis hemopexin determinations are of little value to evaluate the degree of pathologically increased red cell turn over.

### ACKNOWLEDGEMENTS

This investigation was supported in part by grants AM 55558-03 AM 52555 and HD 01919. Dr Oski is a recipient of Carter Development Award No. R3 HD 38600. Dr Lindh had a travel grant No. B68 19R 2486 from the Swedish Medical Research Council.

### REFERENCES

- 1 Augener W. Immunanalysen von Glykoproteinen. In H. Peeters (ed) *Problems of the Biology of Fluids*. Proc. 12th Coll. Elsevier, Amsterdam, 1964, p. 363.
- 2 Bergstrand C. G. Czer B. & Tarutowski P. H. Serum haptoglobin in infancy. *Scand J Clin Lab Invest* 13: 576, 1961.
- 3 Hansson A. & Müller-Eberhard U. Concentrations of serum hemopexin in healthy children and adults and those with a variety of hemolytic disorders. *J Lab Clin Med* 71: 232, 1968.

The haptoglobin concentration paralleled the hemopexin concentration in all these cases although the fluctuations were greater. A sharp fall to very low levels occurred within 24 hours of the exchange transfusion. In one case the haptoglobin concentration remained at this low level while one of the remaining two cases showed a marked and the other a moderate spontaneous increase in the haptoglobin level.

## DISCUSSION

The presence of a heme binding protein among the  $\beta$  globulins has been known for many years (11). In 1960 Nyman showed that only free heme and not hemoglobin could be bound to this protein. Some years later the protein was isolated and given the name of hemopexin and it could be shown that each molecule could bind one molecule of heme (6). The isolation of the protein also meant that specific antisera could be produced to allow quantitation of the protein with different types of immunochemical methods. Using the radial immunodiffusion technique Augener and Muller Eberhard *et al* defined the normal range of hemopexin in different age periods indicating that newborn infants have a hemopexin concentration of approximately 20% of the adult concentration of 50–100 mg/100 ml. Using different methods Sears and Muller Eberhard *et al* tabulated depressed hemopexin levels in diseases with increased red cell turnover in adults and older children. The data of Sears provided support for a theory that the hemopexin level is not markedly depressed until the haptoglobin pool of plasma is depleted. Some support for this theory is obtained also from the data of Muller Eberhard *et al*. In order to better elucidate the relationship between these two proteins immunochemical methods also should be used for the haptoglobin determination since peroxidase methods are very inaccurate at these low levels (8).

With the method used in this study the hemopexin range in a group of healthy young adults was slightly lower than that in the study

of Muller Eberhard *et al*. In spite of this fact the mean value and the range for newborn term infants was slightly higher. This difference may be explained in the present study by the division of the newborn material into term and premature infants. This separation demonstrated a significantly lower hemopexin level among the premature infants compared to the term infants.

The lower values seen in premature and term infants compared to adults may be due either to a lower rate of synthesis or a higher rate of removal. If the hypothesis of Sears that the hemopexin level will not be markedly depressed until all haptoglobin is consumed is correct and if the low hemopexin level seen in hematologically normal newborn infants really was related to consumption secondary to their high red cell turnover then the hemopexin level in the group without haptoglobin should be much lower than in the group with haptoglobin in plasma. The difference however was slight even though statistically significant. This observation together with the circumstance that premature infants had a significantly lower hemopexin level than term infants suggests that the low hemopexin level in hematologically normal infants is primarily a consequence of an immature low synthesis.

If it could be assumed that an exchange transfusion was 100% effective then the fall in the hemopexin level after that procedure should provide some information about the normal turnover rate of the protein. Studies with the aid of serial haptoglobin (4) or carbon monoxide production determinations (9) respectively have shown continuing increased red cell destruction after exchange transfusions. Thus the rapid fall from an increased hemopexin level to a decreased value seen in two of our patients cannot be used in the evaluation of the normal turnover rate.

It has long been known that haptoglobin is absent or present in only small amounts in newborn infants (2, 12, 14) and the results of the present study are consistent with this earlier data. Some of the infants had rather

## THE BLEEDING DISORDER IN HEPATOMEGALIC FORMS OF GLYCOGEN STORAGE DISEASE

INGA MARIE NILSSON and P. A. ÖCKERMAN

From the Coagulation Laboratory, Allmänna Sjukhuset, Malmö, and the Department of Clinical Chemistry, Lundström's Lund, Sweden

A bleeding tendency was found already in the first patients described with glycogen storage disease (GSD) (45-47). Bleeding has since then been seen as a common symptom in patients with hepatomegalic forms of GSD (types I, III and VI).

A more detailed study of the nature of the bleeding disorder has been made by only a few authors. French and German authors, partly in collaboration (1, 18-21), suggested that the bleeding in GSD type I was caused by a thrombopathy due to a deficiency of glucose 6-phosphatase and a storage of large amounts of glycogen in the thrombocytes. In patients with GSD type III and VI no thrombopathy was found (1). Gleichst et al. (13) in a study of eight cases of GSD type I also found a thrombopathy with prolonged bleeding time and reduction in platelet adhesiveness and availability of platelet factor 3.

The existence of a specific glucose 6-phosphatase in normal thrombocytes has, however, not been verified by other authors (17, 35, 43) and the cause of the thrombopathy in GSD type I may therefore need reinvestigation.

The hemostatic defect in GSD type III and VI has not been studied in any detail. It is obvious that the hemostatic defect in these types of GSD cannot be explained by a deficiency of glucose 6-phosphatase in the thrombocytes since in these types lack of glucose 6-phosphatase is not the primary deficiency in the liver.

These considerations formed the basis for the present investigation of the bleeding disorder in patients with the hepatomegalic types I, III and VI of GSD.

## MATERIAL

### *Patients with Glycogen Storage Disease*

#### *Type I (glucose-6-phosphatase deficiency)*

*Case 1 D. T. Female 23* She has never had any definite bleeding symptoms.

*Case 2 A. T. Male 21* He often had nose bleeding from early childhood but after puberty this tendency disappeared. Surgical liver biopsy at age 12 was followed by profuse bleeding.

*Case 3 B. T. Female 17* This patient often has had nose bleeding from early childhood. This tendency is still recurring. She also has profuse menorrhoea. Surgical liver biopsy at age 8 was followed by profuse bleeding.

*Case 4 S. T. Male 9* A slight tendency to nose bleeding started in early childhood.

Cases 1-4 are siblings and have been studied in great detail (4, 31, 34). There remains no doubt about the diagnosis.

*Case 5 E. I. L. Female 11* Published as case 7 in ref. 34. Since early childhood she has a very marked bleeding tendency in the form of nose bleeding, bruising and prolonged bleeding after cuts. The cause of this, no enzymatic studies on liver tissue could be done. The diagnosis GSD type I nevertheless is quite clear because of the findings made in her sister (case 6).

*Case 6 H. L. Female 1* Sister of case 5. Published as case 4 in ref. 22. Glycogen and enzyme assays on liver tissue support the diagnosis. She has no bleeding tendency.

#### *Type III (Amylo 1-6 glucosidase deficiency)*

*Case 7 A. L. Female 12* Published as case 13 in ref. 34. Glycogen and enzyme assays on amniotic tissue

- 4 Heide K, Haupt H, Storik K & Schultze H E. On the heme binding capacity of hemopexin. *Clin Chim Acta* 10: 460 1964
- 5 Kauder E & Mauer A M. Hemolysis as a contributing factor in the bilirubin rebound after exchange transfusion. *J Pediatr* 60: 163 1962
- 6 Koenigsberger M R. Judgement of foetal age I. Neurologic evaluation. *Pediatr Clin N Amer* 13: 835 1966
- 7 Laurell C B. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 15: 45 1966
- 8 Laurell C B & Gronwall C. Haptoglobins. *Advances Clin Chem* 5: 135 1962
- 9 Mausels M J, Pathak A & Nelson N M. Carbon monoxide production and bilirubin excretion in normal and erythroblastotic newborn infants. *Prog Society for Pediatric Res 39th Annual Meeting* 43 1969 (abstr)
- 10 Muller-Eberhard U, Javid J, Liem H H, Hirst A & Hanna M. Plasma concentrations of hemopexin, haptoglobin and heme in patients with various hemolytic diseases. *Blood* 32: 811 1968
- 11 Neale F C, Aber G M & Northam H E. The demonstration of intravascular hemolysis by means of serum paper electrophoresis and a modification of Schumm's reaction. *J Clin Path* 11: 206 1958
- 12 Nyman M. Serum haptoglobin. Methodological and clinical studies. *Scand J Clin Lab Invest* 11 (Suppl 39): 1 1959
- 13 Nyman M. On plasma proteins with heme or hemoglobin binding capacity. *Scand J Clin Lab Invest* 12: 121 1960
- 14 Rausen A R, Gerald P S & Diamond L A. Haptoglobin patterns in cord blood serum. *Nature* 191: 717 1961
- 15 Sears D A. Plasma heme binding in patients with hemolytic disorders. *J Lab Clin Med* 71: 494 1968
- 16 Tarulovskij P H. Quantitative spectrophotometric determination of haptoglobin. *Scand J Clin Lab Invest* 18: 80 1966
- 17 Usher R, McLean F & Scott K E. Judgement of foetal age II. Clinical significance of gestational age and an objective method for its assessment. *Pediatr Clin N Amer* 13: 823 1966

Submitted Sept 18 1969

Accepted Oct 3 1969

(B. L.) Dept of Medicine A  
University Hospital  
Fack  
S-220 05 Lund 5  
Sweden

**Key words** Newborn infants hemolysis hemopexin haptoglobin

Table 1. Bleeding and coagulation tests in patients with glycogen storage disease. Abnormal values are underlined.

	Case 1 (No of in test -5)	Case 2 (No of in test -5)	Case 3 (No of in test -2)	Case 4 (No of in test -2)	Case 5 (No of in test -2)	Case 6 (No of in test -2)	Case 7	Case 8	Case 9 (No of in test -4)	Case 10	Normal range
<i>Bleeding time</i>	4	2-6	2	4-8	1	4	3	4	2-8	4	1-4
<i>Duke's test</i>	5	18-8		14-	>70	11	9	4-15	20	10	6-15
<i>Clot retraction time</i>	14	18	14	14	12	9	—	18	13	12	8-14
<i>Platelet area</i>	25	3	8	4	40	0	—	22	21	19	15-25
<i>Prothrombin consumption</i>	2	12	12	25	31	—	—	—	0	—	0-30
<i>Platelet aggregation test</i>	100 000	140 000	180 000	340 000	340 000	100 000	10 000	340 000	200 000	400 000	135 000- 300 000
<i>Factor VIII activity</i>	34-40	79-40	3-34	40-4	10-32	1-3	34	34	1-32	31-34	38-40
<i>Factor VIII antigen</i>	4-15	26	23	1	3	—	0	—	0-5	—	0-60
<i>Factor IX activity</i>	143	172	—	—	—	—	—	—	100-130	—	90-130
<i>Factor IX antigen</i>	14	14	16	16	15	15	15	14	15	15	14-16
<i>Factor X activity</i>	—	—	100	18	100	100	15	111	115-120	116	80-120
<i>Factor X antigen</i>	10	85	90	15	60	—	—	—	17	—	80-120
<i>Factor XI activity</i>	140	119	138	127	96	14	1.8	127	90-135	145	80-120
<i>Factor XI antigen</i>	—	—	140	137	96	73	0.4	129	125	88	60-140
<i>Thrombin time</i>	2.59	0.8	0.68	0.35	0.43	0.40	0.35	0.36	0.41	0.34	0.25-0.34
<i>Clotting time</i>	19	18	17	7	18	—	20	—	16	—	18-20
<i>Plasma renin</i>	139	11	159	181	95	—	103	—	144	81	60-140
<i>Urea haemolysis test</i>	93	83	83	75	700	—	104	—	164	—	60-140
<i>Fibrinolytic activity</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Activated latex</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Plasma renin</i>	0	0	0	0	0	0	—	—	0	0	0-50
<i>Reninogen activator</i>	0	10	0	25	25	25	—	—	0	0	0-70
<i>Fibrinolytic activity</i>	0	0	0	0	0	—	0	—	—	—	0
<i>mg/100 ml</i>	—	—	—	—	—	—	—	—	—	—	—

support the diagnosis. She has a very moderate bleeding tendency (nose bleeding, bruising) not definitely pathological since early childhood.

**Case 8 I B Male 2** Published as case 2 in ref 22. Glycogen and enzyme assays on liver and muscle tissue support the diagnosis. He has no bleeding tendency.

#### *Type I I (Liver phosphorylase deficiency)*

**Case 9 M II Male 3** Published as case 1 in ref 22. The diagnosis is supported by glycogen and enzyme assays on liver and muscle tissue. He has a moderate bleeding tendency (nose bleeding, bruising, prolonged bleeding after cuts).

**Case 10 T N Female 1** Published as case 3 in ref 22. The diagnosis is supported by glycogen and enzyme assays on liver tissue. She has no obvious bleeding tendency.

## METHODS

**Collection of blood.** The blood was collected with silicone technique and citrated plasma and serum were prepared as described previously (26, 37).

**Coagulation tests.** The following determinations were made: coagulation time in glass and plastic tubes; recalcification time of plasma; prothrombin consumption; prothrombin + factor VII + factor X (Owren's P & II test); factor V AHF (f VIII) time; morpholin B factor (f IX) fibrinogen and thrombin time. The methods used have been described earlier (24, 27). In some patients the prothrombin content in plasma was also determined by an immunochemical method (12).

The bleeding time was performed with Duke's method using standardized haemolets (Dade Reagent Inc. Miami, Florida, U.S.A.). Determinations were performed on both ears. Normal range 1 to 4 min. Use was also made of the method of Ivy as modified by Borchgrevink & Waaler (3, 8, 28). Normal range 5 to 15 min.

Platelet counts were made by the method of Björkman (2).

Platelet adhesiveness was measured according to a slight modification (8) of Hellem's whole blood method (15). Platelet adhesiveness was also measured with the original method of Salzman (39).

Platelet adhesion, aggregation and spreading were studied by phase contrast microscopy of a drop of platelet-rich citrated plasma placed on a glass slide under a cover glass. Platelet aggregation after addition of connective tissue suspension or ADP was studied microscopically and with the aid of a photometer (9).

Clot retraction was determined in diluted platelet-rich plasma according to a modification of Voss method (9).

Platelet factor 3 release was determined according to Hardisty & Hutton (14).

**Fibrinolytic studies.** The following determinations were made: euglobulin clot lysis time; fibrinolytic

activity of plasma and resuspended euglobulin precipitate; plasminogen inhibitors of plasminogen activation by urokinase,  $\alpha$ -macroglobulin and fibrinolytic split products. Unless otherwise stated the methods described earlier were used (10, 23, 29, 37). Plasminogen was determined both by a clot method (34) and by an immunochemical method (11).

## RESULTS

The results of the coagulation studies are given in Table I. *GSD type I* Cases 1-5, all between 9 and 23 years old, had prolonged Ivy bleeding time and three of them had low values for platelet adhesiveness according to Salzman method. There was no decrease of the platelet adhesiveness according to Hellem's method. The platelet factor 3 availability was determined in cases 1 and 2 and was abnormally low in these cases. Platelet count was normal in all except case 5. Four of the patients with *GSD type I* showed increased levels of the factors measured in Owren's P & P test. This finding was supported by immunochemical determination of prothrombin. The plasminogen content was increased in three of the cases. Fibrinogen levels were high in all six patients. The other coagulation factors were mostly within normal ranges. The urokinase inhibitor was high in case 5 but normal in the other cases. There were no signs of increased fibrinolytic activity. Case 6, 1 year old, could not be studied in all details. She had normal Duke and Ivy bleeding time and no signs of a bleeding disorder but she had a high number of platelets.

*GSD type III* Cases 7 and 8 had normal values throughout with the exception of a low platelet adhesiveness according to Salzman's method in case 7, somewhat high platelet count in case 8 and aberrant values for a few coagulation factors. The patient in case 8 was only 2 years old.

*GSD type VI* Case 9, 3 years old, showed the most marked changes in our material. He had somewhat high platelet count, prolonged Duke and Ivy bleeding times, somewhat prolonged coagulation time and decreased platelet adhesiveness both according to Hellem's and





Salzman's method. At examination under the phase contrast microscope his platelets showed a definitely weak aggregation. The platelet factor 3-availability test, however, on repeated occasions showed normal values. This patient also had a high level of prothrombin and fibrinogen. Case 10, one year-old, had normal Ivy bleeding time and normal platelet aggregation, but a high platelet count.

**All patients.** The following values are not included in the table. Clot retraction was normal in all the patients. On direct microscopical examination the platelets appeared normal in all cases. At examination under the phase contrast microscope the platelets adhered to glass and aggregation occurred. But the spontaneous aggregation was delayed in cases 1, 2, 3, 4, 5 and 9. Spontaneous aggregation was normal in cases 8 and 10. In cases 6 and 7 no such investigation was performed. In all cases studied the platelets aggregated after addition of ADP and connective tissue suspension.

### DISCUSSION

Bleeding tendency (44-46), prolonged bleeding time (43) and high number of thrombocytes (40) were the first abnormalities of the hemostatic system found in patients with GSD. No detailed explanation of the nature of the abnormality could be given by these early authors. Schwartz *et al.* (40) found the consumption of prothrombin to be at the lower limit of the normal. Sidbury (41) thoroughly investigated several patients for impaired circulating clotting factors but found no abnormalities. This author therefore considered the bleeding tendency to be caused by altered metabolism of the arteries and veins resulting in delayed or ineffective constriction after injury. Van Creveld (45) found a deficiency of variable clotting factors, sometimes also in the parents.

A qualitative defect of thrombocytes in patients with GSD was first demonstrated by Lelong *et al.* (17). In a more detailed study of six patients with GSD type I by this French group (1) was demonstrated abnormalities of

the bleeding time (prolonged), number of platelets (increased), prothrombin consumption (decreased), thromboelastogram (moderate hypocoagulability) and thrombocyte thromboplastin generation (retarded). Normal findings were reported for capillary resistance, clot retraction, coagulation time and heparin tolerance. In collaboration with German authors (19, 20) it was shown that patients with GSD type I have very high levels of glycogen and a deficiency of glucose 6-phosphatase in the thrombocytes. The conclusion was drawn that these findings might explain the thrombopathy in GSD type I. In four patients with GSD type III and one patient with GSD type VI essentially normal findings were reported except for a high number of thrombocytes. It was concluded that patients with GSD type III and VI did not have a thrombopathy similar to that in GSD type I.

Later authors have put in doubt the existence of a specific glucose 6-phosphatase in normal thrombocytes (16, 34, 42). Also no endoplasmatic reticulum, the structure to which specific glucose 6-phosphatase is firmly bound, could be found in thrombocytes (16).

An increased amount of glycogen in the thrombocytes has been reported not only in GSD type I but also in several other disorders, e.g. Glanzmann's thrombasthenia, von Willebrand's disease, certain malignant and essential thrombocythemas and certain idiopathic chronic thrombocytopenias (references in Alagille *et al.*) (1).

Recently Gulchrest *et al.* (13) published an investigation of eight patients with GSD type I. Five of these (age 12-20) had a bleeding tendency while three (age 1-2) had not. A high platelet count was found in three of the patients, including two of those under 2 years of age. In the five patients over 12 years of age a prolonged bleeding time (according to Ivy's method) and a decreased availability of platelet factor 3 was found. A significant reduction in platelet adhesiveness was found in all eight patients. Glucose infusion or transfusion of normal platelets did not normalize thrombocyte

adhesiveness. The conclusion drawn by Gilchrist *et al* was that "although an intracellular defect may account for the abnormal platelet function environmental plasma factors appear to play a role in the development of the platelet lesion".

Many authors describe a bleeding tendency in their patients already from birth (1, 21, 38, 45). Sidbury (41) in contrast to this considers that the bleeding tendency rarely appears before one year of age but after that age it is common especially in type I but also in type III. We believe that all authors may be right the bleeding tendency sometimes beginning very early and in other cases appearing only after a year or two. Alagille *et al* (1) had a few patients with no platelet abnormality during their first year of life but with a definite such abnormality later.

In our investigation of 6 patients with GSD of type I we found an abnormal platelet function in 5 of them. The one with normal values was a one year old girl. All these patients had a normal platelet adhesiveness according to Hellén's method but decreased according to Salzman's method. It is possible that the values obtained with the Hellén's method are falsely high in these patients. All these patients had a very marked lipaemia. Cronberg & Nilsson (6) have found that infusion of Intralipid in normal individuals causes a marked increase of the platelet adhesiveness as measured by Hellén's method but has no effect on the platelet adhesiveness as measured by Salzman's method. In patients with thrombasthenia Intralipid also caused an increase of the adhesiveness assayed by Hellén's method but Intralipid had no effect on the prolonged bleeding time or the bleeding tendency. The change observed in platelet adhesiveness was therefore believed to be an artifact with no clinical relevance. It was thought that the fat droplets might have changed the properties of the glass surface with consequently increased adhesion of the platelets. The findings of normal platelet count, normal clot retraction, normal Duke bleeding time

prolonged Ivy bleeding time, abnormal platelet factor 3 release, decreased platelet adhesiveness (Salzman), delayed spontaneous platelet aggregation but normal aggregation after addition of connective tissue suspension, thrombin and ADP are similar to those found in patients with mild thrombasthenia (7, 9). Our results concerning the platelet abnormality in GSD type I agree largely with those reported by Gilchrist *et al* (13).

Of interest was the finding that 4 of the 6 patients had increased levels of prothrombin and plasminogen determined both by clot methods and by immunochemical methods. From the report of Gilchrist *et al* it is clear that 5 of their 8 patients had somewhat high prothrombin level determined by Quick's method. These authors did not comment upon this. Lipaemia does not cause increased prothrombin and plasminogen levels (6, 25).

We investigated only two patients with GSD of type III and one of these patients was only 2 years old. Both of them had normal values throughout with the exception of a decreased platelet adhesiveness according to Salzman's method in the older girl. It is known however that even normal individuals can have a low value according to Salzman (5, 39). Thus no definite platelet abnormality was demonstrated in GSD of type III. These patients had neither any bleeding symptoms. Alagille *et al* (1) found no thrombopathy in patients with GSD of type III but they did not study the platelet adhesiveness or platelet aggregation. Our patients with GSD of type III had normal prothrombin levels.

Alagille *et al* (1) also reported that there was no thrombopathy in GSD of type VI. Of our two patients with GSD type VI the one year old boy was completely normal but the 6 years old boy had a marked prolongation of bleeding time and a significant reduction of platelet adhesiveness. It appears probable that our patient with GSD of type VI had the same type of bleeding disorder as the ones with GSD of type I. The patient with GSD type VI had also a high prothrombin level.

We have thus found that patients with GSD of type I and VI have a bleeding disorder which is most similar to that described in patients with mild and/or moderately severe thrombasthenia (7). In the patients that were 1 and 2 years old no definite changes were observed. The biochemical basis for the platelet lesion is unknown.

The practical implications of our findings are important. It is clear that a patient may appear normal in his coagulation bleeding status if only such simple tests are performed as bleeding time according to Duke, platelet count and coagulation time in glass tubes or on a watch glass and if a thorough history is not taken. Nevertheless the patient may have a bleeding disorder. When operations are necessary or in accidental bleeding fresh thrombocyte rich blood taken with silicone or plastic technique or thrombocyte suspensions constitute the best available remedies to normalize the bleeding tendency. The blood substitute Macroderm must not be used since it would increase the bleeding tendency.

### SUMMARY

The bleeding disorder in glycogen storage disease was studied in six patients with type I and two patients each of type III and VI. The most commonly seen abnormalities were prolonged Ivy bleeding time, low values for platelet adhesiveness, increased number of platelets and high values for prothrombin and fibrinogen. It is concluded that patients with type I and VI and possibly also type III may have a bleeding disorder similar to thrombasthenia. When operations are necessary or in accidental bleeding in patients with glycogen storage disease fresh thrombocyte rich blood or thrombocyte suspensions may be needed to normalize the bleeding tendency. The blood substitute Macroderm must not be used since it would increase the bleeding tendency.

### ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (B69 19X 87 05B to IMN and B69 19X 2222 03 to PAO).

### REFERENCES

- 1 Alagille D, Gablan J C & Leloux M Les anomalies plaquettaires au cours de glycoses hépatiques. *Etude de 11 cas*. *Arch Pédiatr Acta* 18 120 1963
- 2 Björkman S E A new method for enumeration of platelets. *Acta Haemat* 22 377 1959
- 3 Borchgrevink C F & Waaler H A The secondary bleeding time. A new method for the differentiation of hemorrhagic diseases. *Acta Med Scand* 162 361 1958
- 4 Brante G, Kasperk & Öckerman P A Glycosenosis type I (lack of glucose 6 phosphatase) in four siblings. *Acta Paediatr Scand Suppl* 157 1964
- 5 Cronberg S Investigations in haemorrhagic disorders with prolonged bleeding time but normal number of platelets with special reference to platelet adhesiveness. *Acta Med Scand Suppl* 486 1968
- 6 Cronberg S & Nilsson I M Coagulation studies after administration of a fat emulsion. *Infusio 2 Thrombus Diathesis Haemorrh* 18 664 1967
- 7 — Investigation of patients with mild thrombasthenia—a haemorrhagic disorder with prolonged bleeding time probably due to a primary platelet defect. *Acta Med Scand* 183 163 1968
- 8 Cronberg S, Nilsson I M & Silver J Studies on the platelet adhesiveness in von Willebrand's disease. *Acta Med Scand* 180 43 1966
- 9 Cronberg S, Nilsson I M & Zetterqvist E Investigation of a family with members with both severe and mild degree of thrombasthenia. *Acta Paediatr Scand* 56 189 1967
- 10 Garrot P O Determination of  $\alpha$ -macroglobulin as trypsin protein esterase. *Clin Chim Acta* 14 493 1966
- 11 Garrot P O & Nitehs J E Immunochemical determination of human plasminogen. *Clin Chim Acta* 22 335 1968
- 12 — Immunochemical determination of human prothrombin. *Scand J Clin Lab Invest* 21 238 1968
- 13 Gilchrist G S, Fine R N & Donnell G N The hemostatic defect in glycogen storage disease type I. *Acta Paediatr Scand* 57 205 1968
- 14 Hardisty R M & Hutton R A The whole clotting time of platelet rich plasma. A test of platelet factor 3 availability. *Brit J Haemat* 11 258 1965
- 15 Hellem A J The adhesiveness of human blood platelets in vitro. *Scand J Clin Lab Invest Suppl* 51 1960
- 16 Huisman F Enzymen bij de periferie bloed bij glycosenose. Thesis Amsterdam 1964
- 17 Lelong M, Alagille D, Gentil C & Gablan J C Glycosenose hépatique par déficit en glu. *Rev Franç Etud Clin Biol* 5 672 1960
- 18 Linnemann P Fortschritte in der Erkennung heterozygoter Mithalitätsfehler bei erblichen Enzymopathien. *Arch B. Schr* 40 553 1962

- 19 Larsson F, Lohr G W, Waller H D & Gross R. Über die Diagnose von Glykogen aus der Glukose 6 Phosphatase Aktivität und dem Glykogengehalt der Thrombocyten. *Enzymol Biol Clin* 133 1962
- 20 — Heterozygoten Test für Glykogenose (v. Gierke's Krankheit). *Acta Medica* 41 352 1963
- 21 Lohr G W. Pathogenesis und Differentialdiagnose der Glykogenosen. *Deutsche Med Wochenschr* 90 1549 1965
- 22 Lundqvist A & Öckerman P A. Fine needle aspiration biopsy of human liver for enzymatic diagnosis of glycogen storage diseases and gangliosidosis. *Acta Paediatr Scand* 1970 in press
- 23 Nilsen J E. Separation and estimation of split products of fibrinogen and fibrin in human serum. *Thrombosis Diathesis Haemorrh* 18 487 1967
- 24 Nilsen J E & Nilsson I M. Demonstration of fibrinolytic split products in human serum by an immunological method in spontaneous and induced fibrinolytic states. *Scand J Haemat* 1 313 1964
- 25 Nilsson I M. Relationen mellan fettmetabolism, blodkoagulation och fibrinolys. *Nervusforskning* 3 66 1960
- 26 Nilsson I M, Blomback M & von Francken I. On an inherited autosomal hemorrhagic diathesis with autohemophilic globulin (AHG) deficiency and prolonged bleeding time. *Acta Med Scand* 159 35 1957
- 27 Nilsson I M, Blomback M & Ranzgren O. Haemophilia in Sweden. I. Coagulation studies. *Acta Med Scand* 170 665 1961
- 28 Nilsson I M, Magnusson S & Borchgrevink Ch. The Duke and Ivy methods for determination of the bleeding time. *Thrombosis Diathesis Haemorrh* 10 223 1963
- 29 Nilsson I M & Olow B. Fibrinolysis induced by streptokinase in man. *Acta Chir Scand* 123 747 1962
- 30 Nilsson I M, Skärne B & Öjdelö A. Fibrinogen in Borch's disease. *Acta Med Scand* 159 463 1957
- 31 Öckerman P A. Phosphorylated glycolytic intermediates in liver and blood cells in members of family with glycogenosis type 3. *Clin Chim Acta* 8 343 1963
- 32 — Glycolysis in plasma in glycogenosis of Cori's type 1. *Clin Chim Acta* 8 910 1963
- 33 — Glucose-6-phosphatase in human jejunal mucosa. Lack of activity in glycogenosis of Cori's type 1. *Clin Chim Acta* 9 151 1964
- 34 — Glycogen storage disease in Sweden. *Acta Paediatr Scand Suppl* 160 1964
- 35 — Glucose glycerol and free fatty acids in glycogen storage disease type 1. Blood levels in the fasting and non fasting state. Effect of glucose and adrenal administration. *Clin Chim Acta* 12 370 1965
- 36 — *In vitro* studies of adipose tissue metabolism of glucose glycerol and free fatty acids in glycogen storage disease type 1. *Clin Chim Acta* 12 383 1965
- 37 Parakevas M, Nilsson I M & Martinsson H. A method for determining serum inhibitors of plasminogen activation. *Scand J Clin Lab Invest* 14 138 1964
- 38 Rømer A, Caldera R, Barrois S & Høllebrand L. Les glycogenoses neonatales. *Arch Franç Pédiat* 17 1 1960
- 39 Salzman E W. Measurement of platelet adhesion. A simple *in vitro* technique demonstrating an abnormality in von Willebrand's disease. *J Lab Clin Med* 62 724 1963
- 40 Schwartz R, Ashmore J & Renold A E. Galactose tolerance in glycogen storage disease. *Pediatrics* 19 485 1957
- 41 Seabury J B Jr. The genetics of the glycogen storage diseases. In: A G Stenberg & A G Beaton (eds). *Progress in Medical Genetics* 4. Grune & Stratton, New York 1965 p 12
- 42 Steiner K. Laboratory diagnosis of glycogen diseases. In: H Sobotta & C P Stewart (eds). *Advances in Clin Chem* 9. Academic Press, New York 1967 p 227
- 43 Unkelin E. Die Glykogenkrankheit. *Jb Kinderheilk* 137 267 1929
- 44 Van Creveld S. Over een bijzondere storing in de koolhydratstofwisseling in den kindertoeftijd. *Nederl Maandschr Geneeskunde* 15 349 1923
- 45 — The clinical course of glycogen disease. *Canad Med Ass J* 88 1 1963
- 46 Von Gierke E. Hepato-nephromegalia glykogenosa (Glykogenose) bei Kindern der Leber und Nieren. *Beitr pathol Anat* 82 497 1929

Submitted Sept 25 1969

Accepted Oct 24 1969

(P A G) Dept of Clinical Chemistry  
Lararvhuset  
S-003 Lund  
Sweden

Key words: Glycogen storage disease, hepatomegaly, bleeding disorders

## SUMMARY

To determine whether intrauterine infections play any part in the aetiology of spina bifida cystica, the immunoglobulin gamma M levels of cord blood from affected children were compared with matched controls. No difference was found. It is concluded that infection does not play a significant role in the aetiology of spina bifida cystica.

## ACKNOWLEDGEMENTS

To the Nursing staff of C Floor for their ready co-operation to Miss Anne Mayfield and Miss Valerie Salter for secretarial assistance and to Mr F Sweetnam for statistical advice. Dr F Albert Recht kindly supplied the pooled serum.

One of us (M A C) was in receipt of a Research Scholarship from the Children's Research Fund.

## REFERENCES

- 1 Alford C A, Schaffer J, Blankinship P W J, Strumfjord J V & Cassady G A. Correlative immunologic, microbiologic and clinical approach to the diagnosis of acute and chronic infections in newborn infants. *New Eng J Med* 277: 437 1967.
- 2 Bernstein L & Wertheim M. *Statistics for Medical and Other Biological Studies*. E & S Livingstone, Edinburgh and London 1952.
- 3 Buckley R H, Dees S C & O'Fallon W M. Serum immunoglobulins—Levels in normal children and in uncomplicated childhood allergy. *Pediatrics* 41: 600 1968.
- 4 Carter C O, Laurence J M & David P A. The genetics of the major central nervous system malformations based on the South Wales Socio genetic Investigation. *Dev Med Child Neurol* 13: 30 1967.
- 5 Eckstein H B. Myelomeningocele basic considerations. *Hospital Medicine* 2: 896 1968.
- 6 Eckstein H B & MacNab G H. Myelomeningocele and hydrocephalus. The impact of modern treatment. *Lancet* 1: 842 1966.
- 7 Gilin D. The differentiation and maturation of specific immune mechanisms. *Acta Paediat Scand* 172: 60 1967.
- 8 Lundstrom R. Rubella during pregnancy. *Acta Paediat Scand* 51: 133 1962.
- 9 Mainland D. *Elementary Medical Statistics*. W B Saunders, London and Philadelphia 1952.
- 10 Mancini G, Carbonara A O & Heremans J F. Immunochemical quantitation of antigens by

single radial immunodiffusion. *Immunochemistry* 2: 235 1960.

- 11 Minson M M, Logan W P D & Loy R. *Bella and Other Virus Infections during Pregnancy*.—Reports on Public Health and Medical Subjects. Her Majesty's Stationary Office 101, Ministry of Health, London 1960.
- 12 Remington J B, Miller M J & Brownlee J. IgM antibodies in acute toxoplasmosis. I. Diagnostic significance in congenital cases and a method for their rapid demonstration. *Pediatrics* 41: 1082 1960.
- 13 Scott A T & Logan L. A specific IgM antibody test in neonatal congenital syphilis. *J Pediatr* 73: 242 1968.
- 14 Sever J & Berendes H W. Cord blood gamma M as a screening test for congenital viral infections. *The Soc Paed Res 37th Annual Meeting*, Pennsylvania 1967.
- 15 Sieber O F Jr, Fulginiti V A, Braze J & Umlauf H J Jr. In utero infection of the fetus by herpes simplex virus. *J Pediatr* 69: 30 1966.
- 16 Southall J F, Hayes B & Dudgeon J A. Immunoglobulins in congenital rubella. *Lancet* 1: 1385 1966.
- 17 St Geme J W Jr & Norr G R. Proposed embryopathic relation between mumps virus and primary endocardial fibroelastosis. *New Eng J Med* 275: 339 1966.
- 18 Stehm R E, Ammann A J & Cherry J D. Elevated cord macroglobulins in the diagnosis of intrauterine infections. *New Eng J Med* 275: 971 1966.
- 19 Stoller A & Collmann R D. Incidence of infectious hepatitis followed by Down's syndrome nine months later. *Lancet* 11: 1221 1965.
- 20 Weller T H & Hanshaw J B. Virological and clinical observations on cytomegalic inclusion disease. *New Eng J Med* 266: 1233 1967.
- 21 West C D, Hong R & Holland N H. Immunoglobulin levels from the newborn period to adulthood and in immunoglobulin deficiency states. *J Clin Invest* 41: 2054 1962.
- 22 van Furth R, Schmit H M E & Hymans W. Immunologic development of the human fetus. *J Exp Med* 122: 1173 1965.

Submitted May 5 1969

Accepted Sept 22 1969

(M A C) Dept of Paediatric Research  
Cardiff Maternity Hospital  
Glossop Terrace  
Cardiff CF1 2XF  
United Kingdom

**Key words** Intrauterine infection, spina bifida cystica, immunoglobulin gamma M level.

## URINARY TESTOSTERONE EXCRETION IN CHILDREN WITH CONGENITAL ADRENAL HYPERPLASIA

O M GAJAL, N M DRAYER and B T RUDD

From the Institute of Child Health University of Birmingham  
Birmingham England

Urinary testosterone excretion in untreated children with congenital adrenal hyperplasia has been reported in a few studies (3-4, 11, 15, 16). In only one instance the study was extended into the newborn period by including a four week-old girl (3).

In the present study four newborn girls and three older patients suffering from congenital adrenal hyperplasia were investigated. The purpose of this study was to see if possible relationships existed between the level of urinary testosterone excretion and indices of virilisation such as fusion of the labia minora in newborn girls and advancement of skeletal maturation in boys.

### METHODS

For the determination of testosterone one tenth of the 4 hour urine collection was hydrolysed with beta glucuronidase and extracted with ether. Chromatography in the system: benzene-ethyl acetate (1:1) (7) light petroleum-dichloro-85% methanol (2:1) (7) was followed by a crystallisation and further chromatography in the system: benzene-ethyl acetate (1:1) (9). Androstenedione was separated from testosterone on the first thin layer plate (9) and epitestosterone was well separated in the paper chromatographic step (4). The final step of the formation of an acetylated product increases the specificity of the method and the separation of the acetylated testosterone from the other products is successfully achieved in the second thin layer step (9).

The amount of testosterone acetate was determined fluorimetrically employing testosterone acetate for the standard curve. 10 µg of testosterone was added to a duplicate urine aliquot and processed

alongside the original sample. The recovery of this sample ranging from 36%-43% was used to correct for losses in the original sample.

One twentieth of the 24-hour urine volume was hydrolysed with beta glucuronidase and extracted with ether-ethyl acetate (2:1). 11-oxy and 11-deoxy 17 ketosteroids were determined spectrophotometrically after paper chromatography in the system: light petroleum-toluene-85% acetic acid (1:1:2) (2). Part of the fraction of medium polarity containing progesterone was crushed with sodium benzoate and then chromatographed in the system: light petroleum-dichloro-85% methanol (1:1:2) (2). The Zimmerman chromogen with mobility similar to authentic androstenedione was eluted and measured spectrophotometrically (6). After subsequent solvent wash of the spent urine 11-deoxy 17 ketosteroids (aliphatic fraction) were also estimated.

Skeletal maturation was assessed according to Greulich & Pyle (7) and fusion of the labia minora according to Prader (12).

### PATIENTS

4/2 seven patients were diagnosed as suffering from congenital adrenal hyperplasia due to 21 hydroxylase deficiency on the basis of clinical and biochemical findings. The patients were normotensive and had not received steroid therapy when they were first seen. Three of the four newborn girls had the salt losing variant of the condition. The three older patients had the non-salt losing variant of the disease (Table 1).

### RESULTS

In the three newborn girls with the salt losing condition the degree of labial fusion corresponded with the level of urinary testosterone excretion (Table 1). However the patient with the smallest degree of labial fusion was studied at the age of 21 days in contrast to the other

## SUMMARY

To determine whether intrauterine infections play any part in the aetiology of spina bifida cystica, the immunoglobulin gamma M levels of cord blood from affected children were compared with matched controls. No difference was found. It is concluded that infection does not play a significant role in the aetiology of spina bifida cystica.

## ACKNOWLEDGEMENTS

To the Nursing staff of C Floor for their ready co-operation, to Miss Anne Mayled and Miss Valerie Salter for secretarial assistance and to Mr P Sweetnam for statistical advice. Dr F Albert Recht kindly supplied the pooled serum.

One of us (M A C) was in receipt of a Research Scholarship from the Children's Research Fund.

## REFERENCES

- 1 Alford C A, Schaffer J, Blankinship P W J, Strumfjord J V & Cassidy G A. Correlative immunologic, microbiologic and clinical approach in the diagnosis of acute and chronic infections in newborn infants. *New Eng J Med* 277 437 1967.
- 2 Bernstein L & Wetherall M. *Statistics for Medical and Other Biological Studies*. E & S Livingstone, Edinburgh and London 1952.
- 3 Buckley R H, Dees S C & O'Fallon W M. Serum immunoglobulins—Levels in normal children and in uncomplicated childhood allergy. *Pediatrics* 41 600 1968.
- 4 Carter C O, Laurence K M & David P A. The genetics of the major central nervous system malformations based on the South Wales Socio-genetic Investigation. *Dev Med Child Neurol* 13 30 1967.
- 5 Eckstein H B. Myelomeningocele: basic considerations. *Hospital Medicine* 2 896 1968.
- 6 Eckstein H B & MacNab G H. Myelomeningocele and hydrocephalus: The impact of modern treatment. *Lancet* 1 842 1966.
- 7 Gitlin D. The differentiation and maturation of specific immune mechanisms. *Acta Paediatr Scand* 172 60 1967.
- 8 Lundstrom R. Rubella during pregnancy. *Acta Paediatr Scand* 51 133 1962.
- 9 Mainland D. *Elementary Medical Statistics*. W B Saunders, London and Philadelphia 1952.
- 10 Mancini G, Carbonara A O & Heremans J F. Immunochemical quantitation of antigens by

single radial immunodiffusion. *Immunochemistry* 2 235 1960.

- 11 Manson M M, Logan W P D & Loy R. *Rubella and Other Virus Infections during Pregnancy*—Reports on Public Health and Medical Subjects. Her Majesty's Stationary Office 101, Ministry of Health, London 1960.
- 12 Remington J S, Miller M J & Brownlee I. IgM antibodies in acute toxoplasmosis: I. Diagnostic significance in congenital cases and a method for their rapid demonstration. *Pediatrics* 41 1082 1960.
- 13 Scotti A T & Logan L. A specific IgM antibody test in neonatal congenital syphilis. *J Pediatr* 73 242 1968.
- 14 Sever J & Berendes H W. Cord blood gamma M as a screening test for congenital viral infections. *The Soc Paed Res* 37th Annual Meeting, Pennsylvania 1967.
- 15 Sieber O F Jr, Fulginiti V A, Braine J & Umlauf H J Jr. In utero infection of the fetus by herpes simplex virus. *J Pediatr* 69 30 1966.
- 16 Southall J F, Hayes K & Dudgeon J A. Immunoglobulins in congenital rubella. *Lancet* 1 1385 1966.
- 17 St Geme J W Jr & Norev G R. Proposed embryopathic relation between mumps virus and primary endocardial fibroelastosis. *New Eng J Med* 275 339 1966.
- 18 Stuehm R E, Ammann A J & Cherry J D. Elevated cord macroglobulins in the diagnosis of intrauterine infections. *New Eng J Med* 275 971 1966.
- 19 Stoller A & Collmann R D. Incidence of infectious hepatitis followed by Down's syndrome nine months later. *Lancet* 11 1221 1965.
- 20 Weller T H & Hamshaw J B. Virological and clinical observations on cytomegalic inclusion disease. *New Eng J Med* 266 1233 1962.
- 21 West C D, Hong R & Holland N H. Immunoglobulin levels from the newborn period to adulthood and in immunoglobulin deficiency states. *J Clin Invest* 41 2054 1962.
- 22 van Furth R, Schmit H M E & Hymans H. Immunologic development of the human fetus. *J Exp Med* 122 1173 1965.

Submitted May 5 1969

Accepted Sept 22 1969

(M A C) Dept of Paediatric Research,  
Cardiff Maternity Hospital,  
Glossop Terrace,  
Cardiff CF1 2XF,  
United Kingdom.

**Key words.** Intrauterine infection, spina bifida cystica, immunoglobulin gamma M level.

Table 2 Urinary testosterone excretion in untreated children with adrenal hyperplasia

Age	Sex	Testosterone (mg/24 hrs)	Authors	Method
years	M	15.4	Tamm <i>et al</i> (1966)	Colorimetric
10 years	M	23.2	Lunn & Duggan (1965)	Fluorimetric
17 years	F	134.0	Vermeulen (1966)	Gas liquid chromatography
13 years	F	216.0	Vermeulen (1966)	Gas liquid chromatography
4 weeks	F	4.8	Camacho & Mignon (1966)	Gas liquid chromatography
6 1/2 years	M	47.3	Camacho & Mignon (1966)	Gas liquid chromatography
14 years	F	86.4	Camacho & Mignon (1966)	Gas liquid chromatography
15 years	F	7.2	Camacho & Mignon (1966)	Gas liquid chromatography

The urinary testosterone and pregnanetriol responses to dexamethasone plus ACTH administration makes an adrenal source for testosterone or its precursor androstenedione highly probable.

The testosterone glucuronide measured in this study is formed peripherally and does not necessarily reflect the amount of testosterone to which the cells in the body are exposed. It could be that inactive precursors of testosterone circulating in the blood are taken up by the periphery i.e. the liver and converted there to testosterone and immediately further metabolized to testosterone glucuronide. Testosterone glucuronide from this source does not indicate the amount of testosterone circulating in blood. However in untreated children with congenital adrenal hyperplasia high urinary testosterone production rates (3-4-10) and also elevated plasma testosterone and androstenedione levels are found (8-10-13). Androstenedione a not very androgenic steroid in man (5) is in equilibrium in blood with testosterone and Horton & Fraser (8) have suggested that in fact almost all of the increased plasma testosterone in congenital adrenal hyperplasia is the result of increased androstenedione secretion by the adrenals.

In the three older patients studied it is difficult to suggest a valid correlation between the level of urinary testosterone excretion and the degree of skeletal advancement. In the newborn girls with the seemingly high urinary testosterone excretion no advancement in skeletal maturation was noted, despite the presence of varying degrees of labial fusion. Possibly more

can be learned from a study of newborn girls who have been exposed *in utero* to progestational agents as in some of these patients both fusion of labia minora and advancement of skeletal maturation at birth have been noted (1).

### SUMMARY

Urinary testosterone 17 ketosteroids and pregnanetriol excretion were determined in seven untreated children with congenital adrenal hyperplasia four of whom were newborn girls with different degrees of fusion of the labia minora.

The level of urinary testosterone excretion correlated with the degree of labial fusion in the three newborn girls with the salt losing variant, and with the degree of advancement of skeletal maturation in the three older children. In the newly born patients the skeletal maturation was not advanced.

In the one patient with the non salt losing variant the urinary testosterone excretion was markedly lower during the thirteenth than the fourth day of life.

### REFERENCES

1. Borchert J, Bongiovanni A M & Eberhart W R. Progestins and skeletal maturation. *New Eng J Med* 268: 215 (1963).
2. Bush I E. *The chromatography of steroids*. Pergamon Press Oxford 1961 p 334.
3. Camacho A M & Mignon C J. Testosterone excretion and production rate in normal adults and in patients with congenital adrenal hyperplasia. *J Clin Endocrinol* 26: 393 (1966).
4. Degenhart, H J, Vimer H B, A., Wilmore R. & Fraumeni L. Production and excretion of



Table 1 Urinary steroid excretion in seven untreated children with congenital adrenal hyperplasia

Age	Sex	Salt loser	Degree of fusion of labia	Bone age	Testosterone $\mu\text{g}/24$ hrs	11 oxy-17 ks	11 deoxy-17 ks		Pregnenetriol $(\text{ng}/24$ hrs)
						glucuronide $(\text{mg}/24$ hrs)	Glucuronide $(\text{mg}/24$ hrs)	Sulphate $(\text{mg}/24$ hrs)	
5 days	F	Yes	III	0	21	12	0.8	3.5	ND
7 days	F	Yes	IV	0	53	0.9	0.8	1.2	0.3
21 days	F	Yes	II	0	12	0.2	0.2	0.6	0.2
4 days					31				
13 days	F	No	IV	0	17				
2½ years	F	No	IV	8 yrs	160	17	1.5	2.1	18.0
3 years	M	No	—	12 yrs	176	3.8	8.7	4.5	2.3
3½ years	M	No	—	10 yrs	89	1.6	1.0	1.9	10.6
				(a)	20	0.4			5.1
				(b)	78	1.6			19.1
4 normal children	3 boys and 1 girl	3½	6 years old	range	1-3	0.7-1.1	0.4-0.6	0.3-0.6	ND

(a) = On 0.25 mg dexamethasone twice daily for 2 months

(b) = On 0.25 mg dexamethasone twice daily for 2 months and 40 I U ACTH Zn for 3 days

ND = Not detected

two patients who were seen at the age of 5 and 7 days. In the only newborn girl with the non salt losing variant where the urinary testosterone excretion could be determined twice during the first weeks of life much less testosterone was excreted on the 13th than on the 4th day of life.

The urinary pregnanetriol excretion in the three newborn girls with the salt losing variant was low (Table 1).

In the three other untreated children all with the non salt losing condition the level of the urinary excretion of testosterone as well as of 17-ketosteroids and pregnanetriol was high (Table 1). There was a marked fall in urinary testosterone and pregnanetriol excretion after two months of steroid therapy (dexamethasone 0.25 mg twice daily) in one of the three patients tested. A rise on the third day of ACTH administration 40 I U ACTH Zn (Organon) whilst the patient was on dexamethasone therapy was also demonstrable (Table 1). In these three older children the advancement in skeletal maturation (Table 1) per year of life was (8 yr-2½/yr) 2½/yr-2.2 (12 yr-3 yr) 3 yr-3 and (10 yr-3½/yr) 3½/yr-1.86 and corresponded with the level of urinary testosterone excretion

which was 160  $\mu\text{g}/\text{d}$  176  $\mu\text{g}/\text{d}$  and 89  $\mu\text{g}/\text{d}$  respectively.

## DISCUSSION

The urinary testosterone excretion corresponded with the degree of labial fusion in the three newborn girls with the salt losing condition. In the one patient with the non salt losing variant where the possible influence of age could be studied a marked drop occurred in the urinary testosterone excretion on the 13th day as compared to the 4th day of life. In older patients the high excretion of urinary testosterone is probably the result of increased stimulation of the adrenal glands (Tables 1 and 2). Although the amount of testosterone excretion in the urine by the four newborn girls was high compared to older children (Table 1) no definite conclusion can be drawn since to our knowledge urinary testosterone excretion levels have not been reported in healthy newborn children.

It is known that in some patients with congenital adrenal hyperplasia the urinary pregnanetriol excretion during the first weeks of life is low and usually after a few months is abnormally high (14).

Table 2 Urinary testosterone excretion in untreated children with adrenal hyperplasia

Age	Sex	Testosterone (mg/24 hrs)	Authors	Method
2 years	M	15.4	Taam et al (1966)	Colorimetric
10 years	M	25.2	Lama & Dagnan (1965)	Fluorimetric
12 years	F	134.0	Vermeulen (1966)	Gas liquid chromatography
13 years	F	216.0	Vermeulen (1966)	Gas liquid chromatography
4 weeks	F	4.8	Camacho & Migeon (1966)	Gas liquid chromatography
6½ years	M	47.3	Camacho & Migeon (1966)	Gas liquid chromatography
14 years	F	86.4	Camacho & Migeon (1966)	Gas liquid chromatography
15 years	F	7.2	Camacho & Migeon (1966)	Gas liquid chromatography

The urinary testosterone and pregnanetriol responses to dexamethasone plus ACTH administration makes an adrenal source for testosterone or its precursor androstenedione highly probable.

The testosterone glucuronide measured in this study is formed peripherally and does not necessarily reflect the amount of testosterone to which the cells in the body are exposed. It could be that inactive precursors of testosterone circulating in the blood are taken up by the periphery i.e. the liver and converted there to testosterone and immediately further metabolized to testosterone glucuronide. Testosterone glucuronide from this source does not indicate the amount of testosterone circulating in blood. However in untreated children with congenital adrenal hyperplasia high urinary testosterone production rates (3-4-10) and also elevated plasma testosterone and androstenedione levels are found (8-10-13). Androstenedione a not very androgenic steroid in man (5) is in equilibrium in blood with testosterone and Horton & Fraser (8) have suggested that in fact almost all of the increased plasma testosterone in congenital adrenal hyperplasia is the result of increased androstenedione secretion by the adrenals.

In the three older patients studied it is difficult to suggest a valid correlation between the level of urinary testosterone excretion and the degree of skeletal advancement. In the newborn girls with the seemingly high urinary testosterone excretion no advancement in skeletal maturation was noted despite the presence of varying degrees of labial fusion. Possibly more

can be learned from a study of newborn girls who have been exposed in utero to progestational agents as in some of these patients both fusion of labia minora and advancement of skeletal maturation at birth have been noted (1).

### SUMMARY

Urinary testosterone 17 ketosteroids and pregnanetriol excretion were determined in seven untreated children with congenital adrenal hyperplasia four of whom were newborn girls with different degrees of fusion of the labia minora.

The level of urinary testosterone excretion correlated with the degree of labial fusion in the three newborn girls with the salt losing variant, and with the degree of advancement of skeletal maturation in the three older children. In the newborn patients the skeletal maturation was not advanced.

In the one patient with the non salt losing variant the urinary testosterone excretion was markedly lower during the thirteenth than the fourth day of life.

### REFERENCES

- Breibart, S., Bongorason, A. M. & Eberheim, W. R. Progestins and skeletal maturation. *New Eng J Med* 268 255 (1963).
- Pash, J. L. *The chromatography of steroids*. Pergamon Press, Oxford 1941 p. 336.
- Camacho, A. M. & Migeon, C. J. Testosterone excretion and production rate in normal adults and in patients with congenital adrenal hyperplasia. *J Clin Endocr* 26 893 (1966).
- Degehorst, H. J., Vreier, H. K., A. Wilms, R. & Frankema, L. Production and secretion of

- testosterone in children with congenital adrenal hyperplasia and precocious puberty in A Vermeulen (ed) *Androgens in normal and pathological conditions* Int Congr Series No 101 Excerpta Medica Foundation Amsterdam 1966 p 81
- 5 Dorfman R I & Shipley R A *Androgen* J Wiley and Sons Inc New York 1956 p 116
  - 6 Epstein E & Zak B A new staining technique for ketosteroid chromatograms *J Clin Endocr* 23 355 1963
  - 7 Greulich W W & Pyle S I *Radiographic atlas of skeletal development of the hand and wrist* Stanford University Press California 1959
  - 8 Horton R & Fraser S D Androstenedione and its conversion to plasma testosterone in congenital adrenal hyperplasia *J Clin Invest* 46 1003 1967
  - 9 Ibayashi H Nakamura M Murakawa S Uchikawa T Tanioka T & Nishio K The determination of urinary testosterone using thin layer chromatography and gas chromatography *Steroids* 3 559 1964
  - 10 Korenman S G Kirschner M A & Lipsett M A Testosterone production in normal and virilised women with the Stern Leventhal syndrome or idiopathic hirsutism *J Clin Endocr* 25 798 1965
  - 11 Lunn N Y & Dingman J I Measurement of testosterone excretion and production rate by glass paper chromatography *J Clin Endocr* 25 563 1965
  - 12 Prader A Der Genitalbefund beim Pseudomaphroditismus femininus des kongenitalen adrenogenitalen Syndroms *Helv Paediatr Acta* 9 231 1954
  - 13 Rivaola M A Saez J M & Migeon C J Studies of androgens in patients with congenital adrenal hyperplasia *J Clin Endocr* 27 674 1967
  - 14 Rudd H T Galal O M & McArthur R O A difficulty in confirming a 21 hydroxylase defect in a neonate *Aust Paediatr J* 4 38 1968
  - 15 Trimm J Apostolakis M & Voight L D The effects of ACTH and HCG on urinary excretion of testosterone in male patients with various endocrine disorders *Acta Endocr (AbA)* 53 61 1966
  - 16 Vermeulen A I *Androgens in normal and pathological conditions* Int Congr Series No 101 Excerpta Medica Foundation Amsterdam 1966 p 71

Submitted April 16 1969

Accepted Oct 13 1969

(O M G) National Research Centre  
Dokki  
Cairo  
U A R

**Key words** Congenital adrenal hyperplasia; urinary testosterone

## AMINO ACID METABOLISM IN HEREDITARY FRUCTOSEMIA

ROLF LINDEMANN LEIV R. GJESSING BRITA MERTON AAGOT CHR. LØKEN  
and SVERRF. HALVORSEN

*From the Paediatric Research Institute Department of Paediatrics the Institute of Pathological Anatomy Rikshospitalet University of Oslo and the Central Laboratory Diakoniværket Hospital Asker Norway*

Hereditary fructosemia is not a relatively benign condition as previously suggested since lethal cases are reported (11, 12, 15). The severity of the symptoms will depend upon the age when fructose ingestion is started.

In early infancy introduction of fructose into the diet will result in vomiting, dystrophy, failure to thrive and hepatorenal dysfunction with generalized aminoaciduria (5). The continued ingestion of fructose may result in cirrhosis of the liver, ascites, edema, brain damage, convulsions and even death (4, 11, 12, 15). The clinical and biochemical findings are similar to the findings in other metabolic diseases (11).

When fructose is included in the diet at a later age the illness is less severe even asymptomatic. Ingestion of fructose results in vomiting or hypoglycemic attacks. Most patients seem to develop a strong aversion to sweets and fruits and thereby protect themselves against the noxious factor (4, 5).

Three cases with symptoms in early infancy and with a lethal course will be presented in this paper. The amino acid metabolism in the acute stage of this disease has not previously been studied in detail and it is the main purpose of the paper to report such studies with special reference to similar studies on patients with acute tyrosinosis.

## METHODS

The plasma and urinary values were obtained by the standard microchemical methods used in our laboratory.

The true blood glucose level was determined by the o-toluidine method (9) and the total blood sugar by that of Hagedorn & Jensen (7). A 20 g/100 ml fructose solution was used in the intravenous fructose tolerance test.

Quantitative determination of serum phenylalanine and tyrosine was performed according to Wong *et al.* (19). Amino acids were determined as serine tetrakis ester and uric acid on a Technicon Amino Acid Analyzer as previously described (16). The phenolic acids were determined simultaneously by paper chromatography (1).

The F1P aldolase<sup>1</sup> and the F1,6-DP aldolase activities in liver homogenates were assayed according to the methods of Wolf (18) and Bruns & Bergmeyer (3).

## CASE REPORTS

**Case 1** J. H. P. a 2770 g boy was born June 12, 1968 after a normal twin pregnancy. The parents were unrelated old r. siblings were normal and there was no history of metabolic disturbances in the family. His dizygotic twin was healthy and weighed 3200 g.

He was breast fed the first week and then placed on a milk formula containing sucrose. Vomiting and loss of weight ensued almost immediately. At age 2 weeks he was hospitalized and treated with frequent meals and methylglutamine without improvement.

On admission to this hospital at the age of 4 weeks he was dystrophic, anorectic and 300 g below his birth weight. His abdomen was distended. A firm liver was

ATP D-fructose 1-phosphotransferase (EC 2.7.1.3)  
Fructose 1,6-diphosphate D-glyceraldehyde 3-phosphate-lyase (EC 4.1.2.4)

Table 1 *Main laboratory findings in three cases of acute hereditary fructosemia*

Patient	Case 1 (J H F)				Case 2 (E G)				Case 3 (B R)		
	Weeks				Weeks				Months		
Age	4	6	6½	p m	4	7/8	21	22	4	5½	6
<i>Blood</i>											
Calcium (mEq/l)	4.6	5.5	3.7	3.1		5.2	5.3		4.3	4.3	
Phosphorus (mg/100 ml)	6.7			7.4		6.6	6.2		2.9	3.2	
Serum protein (g/100 ml)	5.9		4.6	4.0	5.0		6.4	4.9		3.9	5.8
PP index (per cent)	29	14	45	42	5	90	5		44	74	15
Alkaline phosphatase (IU)	125	73		34	175	130	228		135	140	
SGOT (IU)	96	198		135	260	28	500	230	100	88	
SGPT (IU)	93	154		75	340	29		610	65		
Bilirubin (mg/100 ml)	5.0			5.5	11.0	0.9	0.1		1.8		
Blood glucose (mg/100 ml)	42	20	40	10	45		31		74		
Bicarbonate (mEq/l)	26	20	22		13	23	15	17	16	21	18
<i>Urinary reactions</i>											
Protein	-	-	+	+	-	-	+		-	++	+
Benedict	-	+	+	+			+	+	+	+	+
Clinstox	-	-	+	+	-		+		+	+	+
2-4 Dinitrophenylhydrazine	-	-	(+)	-			++	(+)			

p m = post mortem

palpable 2 cm below the costal margin. The only abnormal laboratory findings consisted of a low prothrombin/proconvertin index and slightly elevated serum bilirubin and transaminases. Initially reducing substances could not be demonstrated in the urine (Table 1). Galactosemia was the suspected diagnosis but in spite of a lactose free diet the vomiting continued. At the age of 6 weeks a reducing substance in the urine was identified as fructose.

During this time the patient had recurrent apnoeic episodes requiring artificial ventilation. In addition the patient developed ascites, generalized edema, hyponatremia and hypoglycemia to 14 mg/100 ml at one point. The therapy included human milk feeding and infusions of glucose and bicarbonate but this was to no avail. During an apnoeic episode at 7 weeks of age the patient developed generalized convulsions and died.

At necropsy the liver was moderately enlarged, greenish brown with a finely granular surface and firm consistency. Microscopically extensive periportal fibrosis as well as some centrilobular fibrosis was noted. In addition the liver showed marked proliferation of biliary ducts with bile pigment included in the liver cells often forming pseudocysts.

The kidneys appeared normal macroscopically but showed the proximal convoluted tubules to be slightly distended and to contain eosinophilic material upon microscopy. Deposits of amorphous material which stained positive for calcium were found in and around the cortical tubules and were associated with some degeneration of the epithelial cells.

Macroscopically the brain appeared normal though small with a whitish surface and gelatinous consistency in cut sections. Microscopy showed a pronounced shrinkage of nerve cells with wide pericellular

spaces in the cortex, the striatum and basal ganglia. No apparent reduction in the number of nerve cells had taken place. Some glial nuclei were slightly enlarged but not to the extent described in chronic liver diseases. The white matter of the hemispheres had a loose structure with stellate glial cells containing sudanophilic lipid material in their cytoplasm. A few sudanophilic macrophages were seen around the vessels. Luxol fast blue staining showed myelin to be present in the internal capsule and lower brain stem but absent in the cerebral hemispheres.

Case 2 E G a 3500 g girl was born Sept 8 1968 at term after an uneventful pregnancy. The parents were unrelated and two siblings were normal.

The child was breast fed for the first few days and was then placed on a milk formula containing sucrose. At 2½ weeks she became weak, lost her appetite and vomited after each meal. She was admitted to a local hospital being dystrophic and weighing 300 g below her birth weight. At this time a bulging fontanel was noted.

On admission to this hospital at the age of one month an additional finding was that of a palpable firm liver. A spinal puncture showed a slightly increased pressure and an EEG showed asymmetry and reduced activity over the left temporal region. Following admission the child became weaker, ascites showed an increased bleeding tendency and developed severe metabolic acidosis. The transaminases were markedly increased (Table 1). The proteinuria, melituria and hematuria indicated renal dysfunction. A generalized aminoaciduria with a high excretion of tyrosine and phenolic acids and an elevated serum tyrosine pointed to an acute tyrosinosis. A phenylalanine tyrosine restricted diet containing small amounts of sucrose resulted in a marked clinical im-

improvement with a trend to normalization of liver fraction aminoacidemia and ammoniemia. The child was discharged doing well on a diet containing 80 mg of both phenylalanine and tyrosine per kg per day and 9 g sucrose per day.

She did well at home and her weight increased. At 19 weeks of age she was readmitted for control. The liver was firm and palpable and although the fontanel was still bulging the head circumference was within normal limits. Both serum phenylalanine and tyrosine were normal and a transitory type of tyrosinosis was considered. A phenylalanine loading test was performed and interpreted as normal. At 21 weeks of age she was placed on a normal diet. One day later the serum phenylalanine and tyrosine levels were slightly increased but the normal diet was continued. She then developed anorexia and started to vomit, and a severe metabolic acidosis occurred which was treated with bicarbonate. Besides markedly increased values of serum phenylalanine and tyrosine generalized aminoacidemia, melituria and high excretion of phenolic acids were noted. Again the phenylalanine tyrosine restricted diet was started and she improved. The normal diet contained twice as much phenylalanine and tyrosine as the restricted diet and the sucrose content was 38 g per day compared with the 9 g of the restricted diet. 38 g of sucrose was added to the restricted diet and this produced immediate vomiting and melituria. The blood sugar was found to be 32 mg/100 ml on this sucrose intake. In another period when 38 g lactose was added to the restricted diet no reaction was observed.

A fructose tolerance test was performed (Fig. 1) during which the patient collapsed and glucose had to be given in large amounts. True blood glucose was 15 mg/100 ml confirming the diagnosis of fructosesema.

Phenylalanine and tyrosine were now added to the diet without any deleterious effects and the child was placed on a fructose free diet followed by normalization of the serum and urine values.

Case 3 B.R. a 3600 g girl was born Feb. 6 1963 after an uneventful pregnancy. The parents were unrelated and two siblings were normal.

The child was breast fed for the first 14 days, and was then placed on a milk formula containing sucrose. She was afebrile for some days when three weeks old. At 6 weeks she became febrile and started to vomit shortly after each meal. The tendency to vomit continued and at 4 months she was hospitalized. Dystrophia ossium and an enlarged liver were noted. The only abnormal laboratory findings consisted of a low prothrombin/proconvertin index and increased serum bilirubin and transaminases. In the urine reducing substances and protein were found.

On admission to this hospital some days later she was extremely dystrophic, her abdomen was distended with ascites and a firm liver was palpable 3 cm below the costal margin. Increased serum transaminases, a metabolic acidosis and a low prothrombin/proconvertin index were present (Table 1). Because of its

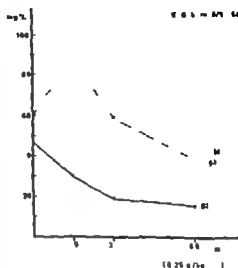


Fig. 1 Intravenous fructose tolerance test in case 2 injected 0.25 g fructose per kg body weight.

tercurrent fever sepsis was considered. Blood cultures gave, however, no evidence for this diagnosis. As galactose was found in the urine she was placed on a lactose free diet, but the red cell galactose-1-phosphate uridylyl transferase level turned out to be normal. The child got worse and repeated abdominal paracenteses were performed followed by plasma transfusions. In addition a generalized aminoacidemia with a particularly high excretion of tyrosine was noted.

In her 7th and 8th months the child clinically improved although hepatomegaly and tyrosinuria were present. The serum amino acids were normal and she was discharged without any definite diagnosis. On reexamination at 10 months she was clinically quite normal. All the laboratory data and the amino acids in both serum and urine were within normal limits. The diagnosis of acute tyrosinosis was supported when the previous data was reexamined but the transient course was not explainable.

On control 6 years after the last admission she was mentally and physically normal. The parents said spontaneously that the girl had developed a strong aversion against all sorts of sweets and fruits. Reexamination of her food intake during the first year of life revealed that she had developed her first symptoms after the milk formula containing sucrose had been given. The clinical improvement came when she was changed from the milk formula to whole milk. Thereafter she protected herself by developing the aversion against sweets.

The anamnestic and clinical data indicated the diagnosis of fructosesema, although liver enzyme studies and fructose tolerance test had not been performed.

UTP =  $\alpha$ -galactose-1-phosphate uridylyltransferase (EC 2.7.1.10).

Table 1 *Main laboratory findings in three cases of acute hereditary fructosemia*

Patient	Case 1 (J H F)				Case 2 (E G)				Case 3 (B R)		
	Weeks				Weeks				Months		
Age	4	6	6½	p m*	4	7/8	21	22	4	5½	6
<i>Blood</i>											
Calcium (mEq/l)	4.6	5.5	3.7	3.1		5.2	5.3		4.3	4.3	
Phosphorus (mg/100 ml)	6.7			7.4		6.6	6.2		2.9	3.2	
Serum protein (g/100 ml)	5.9		4.6	4.0	5.0		6.4	4.9		3.9	5.0
PP index (per cent)	29	14	45	42	5	90	5		44	24	15
Alkaline phosphatase (IU)	125	73		34	175	130	228		135	140	
SGOT (IU)	96	198		135	260	28	500	230	100	88	
SGPT (IU)	81	154		75	340	29		610	88		
Bilirubin (mg/100 ml)	5.0			5.5	11.0	0.9	0.1		1.8		
Blood glucose (mg/100 ml)	42	20	40	10	45		31		74		
Bicarbonate (mEq/l)	26	20	22		13	23	15	17	16	21	18
<i>Urinary reactions</i>											
Protein	-	-	+	+	-	-	+		-	++	+
Benedict	-	+	+	+			+	+	+	+	+
Clinitest	-	-	+	+	-		+		+	+	+
2-4 Dinitrophenylhydrazine	-	-	(+)	-			++	(+)			

\* p m = post mortem

palpable 2 cm below the costal margin. The only abnormal laboratory findings consisted of a low prothrombin/proconvertin index and slightly elevated serum bilirubin and transaminases. Initially reducing substances could not be demonstrated in the urine (Table 1). Galactosemia was the suspected diagnosis but in spite of a lactose free diet the vomiting continued. At the age of 6 weeks a reducing substance in the urine was identified as fructose.

During this time the patient had recurrent apnoeic episodes requiring artificial ventilation. In addition the patient developed ascites, generalized edema, hyponatremia and hypoglycemia to 14 mg/100 ml at one point. The therapy included human milk feeding and infusions of glucose and bicarbonate but this was to no avail. During an apnoeic episode at 7 weeks of age the patient developed generalized convulsions and died.

At necropsy the liver was moderately enlarged, greenish brown with a finely granular surface and firm consistency. Microscopically extensive periportal fibrosis as well as some centrilobular fibrosis was noted. In addition the liver showed marked proliferation of biliary ducts with bile pigment included in the liver cells often forming pseudocysts.

The kidneys appeared normal macroscopically but showed the proximal convoluted tubules to be slightly dilated and in contain eosinophilic material upon microscopy. Deposits of amorphous material which stained positive for calcium were found in and around the cortical tubules and were associated with some degeneration of the epithelial cells.

Macroscopically the brain appeared normal though small with a whitish surface and gelatinous consistency in cut sections. Microscopy showed a pronounced shrinkage of nerve cells with wide pericellular

spaces in the cortex, the striatum and basal ganglia. No apparent reduction in the number of nerve cells had taken place. Some glial nuclei were slightly enlarged but not to the extent described in chronic liver diseases. The white matter of the hemispheres had a loose structure with stellate glial cells containing sudanophilic lipid material in their cytoplasm. A few sudanophilic macrophages were seen around the vessels. Luvol fast blue staining showed myelin to be present in the internal capsule and lower brain stem but absent in the cerebral hemispheres.

Case 2 E G, a 3500 g girl was born Sept 8 1968 at term after an uneventful pregnancy. The parents were unrelated and two sibs were normal.

The child was breast fed for the first few days and was then placed on a milk formula containing sucrose. At 2 1/2 weeks she became weak, lost her appetite and vomited after each meal. She was admitted to a local hospital being dystrophic and weighing 500 g below her birth weight. At this time a bulging fontanel was noted.

On admission to this hospital at the age of one month an additional finding was that of a palpable firm liver. A spinal puncture showed a slightly increased pressure and an EEG showed asymmetry and reduced activity over the left temporal region. Following admission the child became weaker, ascitic and showed an increased bleeding tendency and developed severe metabolic acidosis. The transaminases were markedly increased (Table 1). The proteinuria, mellituria and hematuria indicated renal dysfunction. A generalized aminoaciduria with a high excretion of tyrosine and phenolic acids and an elevated serum tyrosine pointed to an acute tyrosinosis. A phenylalanine tyrosine restricted diet containing small amounts of sucrose resulted in a marked clinical im-

provement with a trend to normalization of liver fraction aminoaciduria and aminoaciduria. The child was discharged doing well on a diet containing 20 mg of both phenylalanine and tyrosine per kg per day and 9 g sucrose per day.

She did well at home and her weight increased. At 19 weeks of age she was readmitted for control. The liver was firm and palpable and although the fontanel was still bulging the head circumference was within normal limits. Both serum phenylalanine and tyrosine were normal and a transitory type of tyrosinosis was considered. A phenylalanine loading test was performed and interpreted as normal. At 21 weeks of age she was placed on a normal diet. One day later the serum phenylalanine and tyrosine levels were slightly increased but the normal diet was continued. She then developed sickness and started to vomit, had a severe metabolic acidosis occurred which was treated with bicarbonates. Besides markedly increased values of serum phenylalanine and tyrosine generalized aminoaciduria mellinosis and high excretion of phenolic acids were noted. Again the phenylalanine tyrosine restricted diet was started and she improved. The normal diet continued twice as much phenylalanine and tyrosine as the restricted diet and the sucrose content was 38 g per day compared with the 9 g of the restricted diet. 38 g of sucrose was added to the restricted diet and the produced immediate vomiting and mellaemia. The blood sugar was found to be 32 mg/100 ml on this sucrose intake. In another period when 38 g lactose was added to the restricted diet no reaction was observed.

A fructose tolerance test was performed (Fig. 1) during which the patient collapsed and glucose had to be given in large amounts. True blood glucose was 13 mg/100 ml confirming the diagnosis of fructosemia.

Phenylalanine and tyrosine were now added to the diet without any deleterious effects and the child was placed on a fructose free diet followed by normalization of the serum and urine amino acids.

Case J. B. R. a 1600 g girl was born Febr. 6 1963 after an uneventful pregnancy. The parents were unrelated and two siblings were normal.

The child was breast fed for the first 24 days and was then placed on a milk formula containing sucrose. She was sick for some days when three weeks old. At 6 weeks she became feverish and started to vomit shortly after each meal. The tendency to vomit continued and at 4 months she was hospitalized. Dystrophias, parasites and an enlarged liver were noted. The only abnormal laboratory findings consisted of a low prothrombin/proconvertin index and increased serum bilirubin and transaminases. In the urine reducing substances and protein were found.

On admission to the hospital some days later she was extremely dystrophic, her abdomen was distended with ascites and a firm liver was palpable. 3 cm below the costal margin increased serum transaminases, a metabolic acidosis and a low prothrombin/proconvertin index were present (Table 1). Because of an

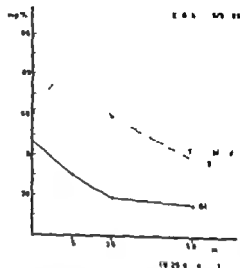


Fig. 1 Intravenous fructose tolerance test in case 2 injected 0.25 g fructose per kg body weight.

intercurrent fever sepsis was considered. Blood cultures gave however no evidence for this diagnosis. As galactose was found in the urine she was placed on a lactose free diet but the red cell galactose 1 phosphate uridylyl transferase level turned out to be normal. The child got worse and repeated abdominal paracenteses were performed followed by plasma transfusions. In addition a generalized aminoaciduria with a particularly high excretion of tyrosine was noted.

In her 7th and 8th months the child clinically improved although hepatomegaly and tyrosinuria were present. The serum amino acids were normal and she was discharged without any definite diagnosis. On readmission at 10 months she was clinically quite normal. All the laboratory data and the amino acids in both serum and urine were within normal limits. The diagnosis of acute tyrosinosis was suggested when the previous data was reexamined but the transient course was not explainable.

On control 6 years after the last admission she was mentally and physically normal. The parents and spontaneously that the girl had developed a strong aversion against all sorts of sweets and fruits. Recollection of her food intake during the first year of life revealed that she had developed her first symptoms after the milk formula containing sucrose had been given. The clinical improvement came when she was changed from the milk formula to whole milk. Thereafter she protected herself by developing the aversion against sweets.

The anamnestic and clinical data indicated the diagnosis of fructosemia although liver enzyme studies and fructose tolerance test had not been performed.

UTP = D-galactose 1 phosphate uridylyltransferase (EC 2.7.7.10).



Table 1 *Main laboratory findings in three cases of acute hereditary fructosemia*

Patient	Case 1 (J H F)				Case 2 (E G)				Case 3 (B R)		
	Weeks				Weeks				Months		
Age	4	6	6½	p m	4	7/8	21	22	4	5½	6
<i>Blood</i>											
Calcium (mEq/l)	4.6	5.5	3.7	3.1		5.2	5.3		4.3	4.3	
Phosphorus (mg/100 ml)	6.7			7.4		6.6	6.2		2.9	3.1	
Serum protein (g/100 ml)	5.9		4.6	4.0	5.0		6.4	4.9		3.9	5.0
PP index (per cent)	29	14	45	42	5	90	5		44	74	15
Alkaline phosphatase (IU)	125	73		34	175	130	228		135	140	
SGOT (IU)	96	198		135	260	28	500	230	100	88	
SGPT (IU)	93	154		75	340	29		610	65		
Bilirubin (mg/100 ml)	5.0			5.5	11.0	0.9	0.1		1.8		
Blood glucose (mg/100 ml)	42	20	40	10	45		31		74		
Bicarbonate (mEq/l)	26	20	22		13	23	15	17	16	21	11
<i>Urinary reactions</i>											
Protein	-	-	+	+	-	-	+		-	++	+
Benedict	-	+	+	+			+	+	+	+	+
Clinitest	-	-	+	+	-		+		+	+	+
2-4 Dimethylhydrazine	-	-	(+)	-			++	(+)			

p m = post mortem

palpable 2 cm below the costal margin. The only abnormal laboratory findings consisted of a low prothrombin/proconvertin index and slightly elevated serum bilirubin and transaminases. Initially reducing substances could not be demonstrated in the urine (Table 1). Galactosemia was the suspected diagnosis but in spite of a lactose free diet the vomiting continued. At the age of 6 weeks a reducing substance in the urine was identified as fructose.

During this time the patient had recurrent apnoeic episodes requiring artificial ventilation. In addition the patient developed acute generalized edema by hyponatremia and hypoglycemia to 14 mg/100 ml at one point. The therapy included human milk feeding and infusions of glucose and bicarbonate but this was to no avail. During an apnoeic episode at 7 weeks of age the patient developed generalized convulsions and died.

At necropsy the liver was moderately enlarged, greenish brown with a finely granular surface and firm consistency. Microscopically extensive periportal fibrosis as well as some centrilobular fibrosis was noted. In addition the liver showed marked proliferation of biliary ducts with bile pigment included in the liver cells often forming pseudocysts.

The kidneys appeared normal macroscopically but showed the proximal convoluted tubules to be slightly distended and to contain eosinophilic material upon microscopy. Deposits of amorphous material which stained positive for calcium were found in and around the cortical tubules and were associated with some degeneration of the epithelial cells.

Macroscopically the brain appeared normal though small with a whitish surface and glutinous consistency in cut sections. Microscopy showed a pronounced shrinkage of nerve cells with wide pericellular

spaces in the cortex, the striatum and basal ganglia. No apparent reduction in the number of nerve cells had taken place. Some glial nuclei were slightly enlarged but not to the extent described in chronic liver diseases. The white matter of the hemispheres had a loose structure with stellate glial cells containing sudanophilic lipid material in their cytoplasm. A few sudanophilic macrophages were seen around the vessels. Luxol fast blue staining showed myelin to be present in the internal capsule and lower brain stem but absent in the cerebral hemispheres.

Case 2. G, a 3500 g girl was born Sept. 8, 1968 at term after an uneventful pregnancy. The parents were unrelated and two siblings were normal.

The child was breastfed for the first few days and was then placed on a milk formula containing sucrose. At 2½ weeks she became weak, lost her appetite and vomited after each meal. She was admitted to a local hospital, being dystrophic and weighing 500 g below her birth weight. At this time a bulging fontanel was noted.

On admission to this hospital at the age of one month an additional finding was that of a palpable firm liver. A spinal puncture showed a slightly increased pressure and an EEG showed asymmetry and reduced activity over the left temporal region. Following admission the child became weaker, afebrile, showed an increased bleeding tendency and developed severe metabolic acidosis. The transaminases were markedly increased (Table 1). The proteinuria, melluria and hematuria indicated renal dysfunction. A generalized ammoniaciduria with a high excretion of tyrosine and phenolic acids and an elevated serum tyrosine pointed to an acute tyrosinosis. A phenylalanine tyrosine restricted diet containing small amounts of sucrose resulted in a marked clinical im-

Table 4 Excretion of the urinary amino acids and the phenolic acids in three cases of acute hereditary fructosemia expressed as  $\mu\text{moles/mg creatinine}$ 

	Normal* 11 wks	Case 1 6½ wks	Case 2 4 wks	Case 3 4 months
aspartoamino acid	0.02-0.33	1.14	0.90	0.82
serine	0.63-3.45	1.58	2.10	0.36
lactosamine sulphate	0	0.86	0.40	0.15
hydroxyproline	0.56-0.93	11.30	6.00	5.80
seric acid	0-0.10		6.18	5.18
lysine	0.54-0.58	46.37	26.00	36.96
alanine	1.10-1.64	Large	14.90	37.65
leucine	0.04-0.21	1.72	4.90	2.10
valine	0.04-0.21	33.63	37.20	10.32
isoleucine	0	3.82	6.60	4.95
tyrosine	2.60-2.79	Large	75.90	40.79
methionine	1.06-1.37	35.83	72.90	27.49
phenylalanine	0-0.24	0.47	0.80	0.79
histidine	0.02-0.27	5.67	9.90	2.70
proline	0.22	2.50	2.00	1.30
lysine	Tr-0.8	5.45	4.80	1.58
cystathionine	0	1.57	2.70	0.69
serine	Tr-0.16	0.63	1.20	0.22
leucine	Tr-0.20	2.31	3.20	1.57
tyrosine	0.24-0.37	16.10	25.90	10.92
phenylalanine	0-0.16	10.52	6.80	4.54
3-aminobutyric acid	0.89-2.94	2.96	0.76	0.66
ethanolamine	0.41-0.62	5.71	5.40	2.60
hydroxytyrosine	0	0.23	0.10	0.15
2,4-diaminobutyric acid		2.56	2.20	1.14
oxalate	Tr-0.12			
lysine	0.36-0.58			
1-methylhistidine	0.08	19.45	11.70	9.96
histidine	1.25-1.82			
3-methylhistidine	0.14-0.18	41.22	21.70	18.34
carotene	0.38-0.40	0.16	0.36	Tr
arginine	0.07-0.10	0.16	0.03	0.15
p-hydroxy phenyl lactic acid		31.5	33.5	4.4
p-hydroxy phenyl acetic acid		3.9	23.0	2.6
tyrosine		16.1	25.5	10.9

\*Arneberg *et al.* (2)

In case 2 the excretion of tyrosine was about 70 times the normal phenylalanine about 40 times and proline about 180 times. The tyrosine and phenylalanine excretion in case 3 was each about 30 times the normal and proline about 50 times.

The excretion of the phenolic acids *p*-hydroxy phenyl lactic acid and *p*-hydroxy phenyl acetic acid was markedly elevated in cases 1 and 2 and slightly elevated in case 3 (Table 4).

A phenylalanine tolerance test was performed in case 2 when she was ingested a small amount of sucrose. The serum phenylalanine increased to a maximum of 8.1 mg/100 ml after 30 min and the serum tyrosine to a maxi-

mum of 2.6 mg/100 ml. The findings were interpreted as normal.

## DISCUSSION

The diagnosis of fructosemia was suggested in case 1 when the patient collapsed, was hypoglycemic and had fructosuria after ingestion of fructose. Cases 2 and 3 were diagnosed on the basis of clinical and laboratory evidence and the improvement on a therapeutic trial.

These cases of fructosemia in early infancy illustrate that the onset and severity of the symptoms are determined by the age at which fructose is introduced into the diet. The symptoms do not necessarily occur immediately but

Table 2 *Enzyme activity in liver homogenate in a case of acute hereditary fructosemia expressed as  $\mu\text{moles/g/hour}$*

	F 1 P aldolase	F 16-DP aldolase	F 16-DP aldolase/ aldolase
Case 1 (J H F)	43.6	394.4	9.25
Normal liver	623.6	782.7	1.25

### LABORATORY INVESTIGATIONS

All three cases had normal serum electrolytes determined on several occasions, except for the periods of collapse after fructose ingestion. They had low plasma protein levels and developed metabolic acidosis following prolonged ingestion of fructose.

Icterus was observed in cases 1 and 2 and was reported in case 3. They all had a slightly elevated alkaline phosphatase and a low prothrombin/proconvertin index which increased after vitamin K injections. The transaminases SGOT and SGPT were elevated in all three cases and were particularly high during an acute episode in case 2. All values were normalized on a fructose free diet.

In cases 1 and 2, serum phosphorus was not determined immediately after fructose ingestion and was found to be within normal limits or in the upper range of normal. In case 3 however the serum phosphorus levels were low increasing as the patient improved.

The blood sugar was found markedly decreased immediately after fructose ingestion in cases 1 and 2. Severe hypoglycemia occurred following intravenous fructose loading in case 2.

**Liver enzymes** Enzymatic activity in liver was assayed in case 1 only (Table 2). The liver was removed immediately after death and kept frozen ( $-20^{\circ}\text{C}$ ) until investigated. The ratio F 16 DP aldolase/F 1 P aldolase which normally should be 1.0 was found to be 9.25 in our case. To exclude a reduction of the F-1 P aldolase secondary to a generalized liver disease, the  $\beta$  glucuronidase<sup>1</sup> was determined (17) and found to be within normal limits.

<sup>1</sup>  $\beta$ -D-glucuronide glucuronohydrolase (EC 3.2.1.31)

**Serum and urinary amino acids** In all three cases generalized aminoacidemia (Table 3) and generalized aminoaciduria (Table 4) were found. In case 3 only ascitic fluid and not serum was investigated but it is assumed that ascitic fluid is equilibrated with serum. For the results of the individual amino acids see Tables 3 and 4.

In case 1 the serum tyrosine was about 3 times the normal and methionine about 16 times. The serum tyrosine in case 2 was about 1 1/2 times the normal and methionine about 2 times. In case 3 the amino acids in the ascitic fluid were within normal limits except for methionine which was about 6 times the normal.

The urinary excretion of tyrosine in case 1 was about 40 times the normal, phenylalanine about 65 times and proline about 160 times.

Table 3 *Serum amino acids in two cases and ascitic fluid amino acids in one case of acute hereditary fructosemia expressed as  $\mu\text{mol/liter}$*

	Normal <sup>a</sup> 12 wks	Case 1 6½ wks	Case 2 4 wks	Case 3 <sup>b</sup> 4 months
Taurine	74-216	401	197	40
Methionine		25	7	17
Sulfuric acid		150	70	
Hydroxyproline		18	77	
Aspartic acid	Tr - 17	615	233	65
Threonine	114-335	888	463	143
Serine	94-243	135	309	31
Glutamic acid	20-107	420	387	198
Proline	107-277	110	68	41
Citrulline	9-29	2468	806	97
Glycine	224-514	865	788	212
Alanine	236-410			
$\alpha$ amino acid				
butyric acid	6-29	46	19	10
Valine	80-246	192	177	73
Cysteine	35-84	124	26	28
Methionine	9-41	664	70	239
Cystathionine		28	26	3
Isoleucine	27-53	67	53	26
Leucine	47-109	134	121	42
Tyrosine	42-99	316	113	84
Phenylalanine	42-110	177	91	65
Ethanolamine	26-92	282	40	16
Ornithine	49-151	288	131	34
Lysine	114-269	576	292	102
Histidine	49-114	166	228	39
Arginine	27-88	168	57	51

<sup>a</sup> Perry et al (14)

<sup>b</sup> Ascitic fluid

sacc test has been performed which gave a normal rise in both blood glucose and fructose.

The findings presented in this paper support the view that the clinical and biochemical picture of acute tyrosinosis may arise from different metabolic disorders. Furthermore they support the concept that the hepatorenal dysfunction observed in tyrosinosis or hereditary tyrosinemia is secondary to some other metabolic defect than one in the tyrosine pathway.

## SUMMARY

Three patients with hereditary fructosemia are presented and discussed, one with a lethal course in the acute form of fructosemia after prolonged administration of fructose. Infants may show similar clinical laboratory and histological findings as infants with "acute tyrosinosis". Both disorders exhibit hepatomegaly, edema & cites generalized aminoaciduria and striking excretion of phenolic acids. These findings support the view that the clinical and biochemical picture of acute tyrosinosis may arise from different metabolic disorders. Furthermore they support the concept that the hepatorenal dysfunction observed in tyrosinosis or hereditary tyrosinemia is secondary to some other metabolic defect than one in the tyrosine pathway.

## REFERENCES

1. Armstrong M D, Shew K N & Wall P E. The phenolic acids of human urine: paper chromatography of phenolic acid. *J Biol Chem* 195 793 1956
2. Armstrong M D, Yates K N & Connolly J P. Amino acid excretion of newborn infants during the first twenty-four hours of life. *Pediatr* 1955 13 975 1964
3. Brown F H & Bergmeyer H U. Fructose 1,6-diphosphate aldolase. In H U Bergmeyer (ed) *Methods of enzymatic analysis*. Academic Press New York 1965 p 724
4. French E R, Prader A, Labhart A, Seaber H W & Wolf H P. Die hereditäre Fructoseintoleranz, eine bisher nicht bekannte Stoffwechselerkrankung. *Schweiz Med Wochschr* 87 1168 1957
5. French E R, Wolf H P, Bartsch H, Prader A & Labhart A. Hereditary fructose intolerance. *Am J Med* 34 151 1963
6. Gentz J, Jørgensen R & Zetterstrom R. Tyrosinemia. *J Pediatr* 66 670 1965
7. Hagström, H C, Halström F & Jensen N. Hurtige metoder til bestemmelse av blod sukker ved kaliumferricyanid. *Hospitaltidn* 78 1193 1935
8. Halvorsen S, Pande H, Loken Aa C & Gjerming L R. Tyrosinosis. *Arch Dis Child* 41 238 1966
9. Hultman E. Rapid specific method for determination of aldosesaccharides in body fluids. *Nature* 183 108 1959
10. Lalow M, Alagille D, Gentz C, Colin J, Mercier C, Tuben J & Bouquyer J. L'intolérance héréditaire au fructose. *Arch Franc Pediatr* 19 841 1962
11. Levin B, Soodgran G J, Oberholzer V G, Burgess E A & Dobbs R. II. Fructosemia. *Am J Med* 45 826 1968
12. Lindemann R, Gysberg L R, Merion B & Halvorsen S. Fructosemia ("Acute tyrosinosis"). *Lancet* 1 891 1969
13. Odellro M, Gentz M & Røn E. Intolerance héréditaire au fructose du nourisson. *Arch Franc Pediatr* 26 413 1969
14. Perry T L, Hardwick D F, Dixon G H, Dolman C L & Hansen S. Hypertyrosinemia. A metabolic disorder associated with cartilage inlet cell hyperplasia and renal tubular degeneration. *Pediatrics* 36 236 1965
15. Saenz R, Joffe J, Metay P, Sordani J & Dorel N. Un cas mortel d'intolérance héréditaire au fructose. *Pediatrics* 17 875 1962
16. Seip M, Stein Johnsen J, Vellan J E & Gjerming L R. Dietary treatment of cystinosis. *Acta Paediatr Scand* 57 409 1968
17. Wikstrom A. Studies of the activation *in vitro* of glucosyltransferase. *Biochim Biophys Acta* 191 297 1969
18. Wolf H P. 1 Phosphofructaldolase. In H U Bergmeyer (ed) *Methods of enzymatic analysis*. Academic Press New York 1965 p 732
19. Wong P W K, O'Flynn M D & Isouye T. Micromethods for measuring phenylalanine and tyrosine in serum. *Clin Chem* 10 1093 1964

Submitted Sept. 2 1969

Accepted Oct. 29 1969

(R. L.) Dept. of Paediatrics  
Rikshospitalet  
Oslo 1  
Norway

*Key words:* Fructosemia, acute tyrosinosis, amino acids, newborn error of metabolism, pathological and anatomical findings.

may develop gradually one or more weeks after the regular ingestion of fructose

The acute form of fructosemia in early infancy may show the same clinical and biochemical picture as acute tyrosinosis (8, 12). Both conditions may cause hepatomegaly, ascites, edema, pathological liver function tests, proteinuria, mellituria and SGOT and SGPT elevation. In our cases these were more marked in fructosemia than in tyrosinosis.

In fructosemia, the serum phosphorus usually decreases after ingestion of fructose. In our cases phosphorus was not determined immediately after fructose ingestion and was found at the upper limit of normal in cases 1 and 2 and moderately low in case 3. Even though considerable phosphaturia may occur (11) rickets have not been described in fructosemia although this is a constant finding in tyrosinosis (6, 8). The reason may be that patients with undiagnosed fructosemia die before rickets develops or that renal tubular function is normalized in those who develop an aversion to sweets.

The serum amino acids are elevated both in fructosemia and acute tyrosinosis but in fructosemia aminoacidemia appears to be more generalized. The methionine level is very high in both disorders in the acute stage and tyrosine is elevated particularly in acute tyrosinosis. A marked phenolic acid excretion and generalized aminoaciduria are found in both disorders with proline excretion being particularly high in fructosemia and only moderately increased in acute tyrosinosis. In the cases with fructosemia the tyrosyluria disappeared as fructose was removed from the diet.

A study of the liver in case 1 showed cholestasis, stenosis of the hepatic duct and probable obliteration of the cystic duct. The stenosis may account for some of the hepatic fibrosis and biliary proliferation but the picture is similar to that described previously in fructosemia (10, 13, 15). Degeneration of the renal tubular epithelium has been described in fructosemia while associated calcified deposits have not been reported.

The findings of retarded myelination and lipid storage in the glial cells of the brain seem to indicate that the metabolic derangement may be interfering with myelination, a very active process during this period of development.

Comparing the histological findings with those described in tyrosinosis (8) there are also striking similarities. Signs of regenerating liver parenchyma which are pronounced in tyrosinosis were lacking in the present case. It should however be remembered that case 1 was only 7 weeks old, while cases of tyrosinosis previously examined have all died at later ages.

Mental changes in tyrosinosis have been observed clinically (6). Sudanophilic granules in glial cells were observed in one out of the three cases of tyrosinosis (8). The picture of enlarged naked glial nuclei or liver glia in cortex and striatum that dominated the picture in those three cases, was not prominent in the present case. Such changes are however usually combined with more prolonged cases of liver diseases. Considering individual differences and age differences the findings described in tyrosinosis and fructosemia both in liver, kidneys and brain are closely related.

In fructosemia the marked derangement in tyrosine metabolism occurring after fructose ingestion judging from the excreted metabolites is probably due to the decreased activity of *p*-hydroxyphenylpyruvate hydroxylase<sup>1</sup>. The activity of this enzyme is most easily influenced during infancy. There is also a derangement in methionine metabolism, as in acute tyrosinosis. The proline excretion should be investigated in future cases of fructosemia.

Based on our present experience it is not possible to make a differential diagnosis between fructosemia and acute tyrosinosis on the basis of the urine amino acid excretion, serum amino acids and the phenolic acid excretion when the child is on a normal diet. A fructose tolerance test must be performed to make a definite diagnosis. In two of our chronic cases of tyrosinosis a fructose tolera-

<sup>1</sup> (EC 1.99.1.14)

test has been performed which gave a normal rise in both blood glucose and fructose.

The findings presented in this paper support the view that the clinical and biochemical picture of acute tyrosinosis may arise from different metabolic disorders. Furthermore they support the concept that the hepatorenal dysfunction observed in tyrosinosis or hereditary tyrosinemia is secondary to some other metabolic defect than one in the tyrosine pathway.

### SUMMARY

Three patients with hereditary fructosemia are presented and discussed, one with a lethal course. In the acute form of fructosemia after prolonged administration of fructose infants may show similar clinical laboratory and histological findings as infants with acute tyrosinosis. Both disorders exhibit hepatomegaly, edema, ascites, generalized aminoaciduria and striking excretion of phenolic acids. These findings support the view that the clinical and biochemical picture of "acute tyrosinosis" may arise from different metabolic disorders. Furthermore they support the concept that the hepatorenal dysfunction observed in tyrosinosis or hereditary tyrosinemia is secondary to some other metabolic defect than one in the tyrosine pathway.

### REFERENCES

- 1 Armstrong M D, Shaw K N & Wall P E. The phenolic acids of human urine: paper chromatography of phenolic acid. *J Biol Chem* 218: 293 1956.
- 2 Armstrong M D, Yates K N & Connelly J P. Amino acid excretion of newborn infants during the first twenty-four hours of life. *Pediatr* 1967 33: 975 1964.
- 3 Bryan F H & Bergmeyer H U. Fructose 1,6-diphosphate aldolase. H U Bergmeyer (ed.) *Methods in enzymatic analysis*. Academic Press, New York 1965 p 724.
- 4 Froesch E R, Prader A, Labhart A, Stuber H W & Wolf H P. Die hereditäre Fructoseintoleranz, eine bisher nicht bekannte Stoffwechselerkrankung. *Schweiz Med Wochschr* 87: 1168 1957.
- 5 Froesch E R, Wolf H P, Bartsch H, Prader A & Labhart A. Hereditary fructose intolerance. *Amer J Med* 34: 151 1963.
- 6 Gentz J, Jägersburg R & Zetterstrom R. Tyrosinemia. *J Pediatr* 66: 670 1965.
- 7 Hagström H C, Halstrom F & Jensen N. Hurtige metoder til bestemmelse av blodmikter ved kaliumferricyanid. *Hospitalslind* 78: 1193 1935.
- 8 Halvorsen S, Færevik H, Loken Aa C & Gjessing L R. Tyrosinosis. *Arch Dis Child* 41: 238 1966.
- 9 Hultman L. Rapid specific method for determination of aldose-saccharides in body fluids. *Nature* 183: 106 1959.
- 10 Lelon M, Abgrille D, Gentz C., Colin J, Mercier C, Tubin J & Bouquier J. L'Intolérance héréditaire au fructose. *Arch Franc Pediatr* 19: 841 1962.
- 11 Levin B, Seodgrass G J, Oberholzer V E, Burgess E A & Dobbs R H. Fructosemia. *Amer J Med* 45: 826 1968.
- 12 Landemann R, Gysmans L, R. Merton B & Halvorsen S. Fructosemia/Acute tyrosinosis. *Lancet* 1: 891 1969.
- 13 Odehne M, Gieseler M & Riet D. Intolérance héréditaire au fructose du sucrose. *Arch Franc Pediatr* 26: 435 1969.
- 14 Perry T L, Hardwick D F, Dixon M H, Dolman, C L & Hensen, S. Hypermethioninemia: A metabolic disorder associated with cartilage, skin, cell hyperplasia and renal tubular degeneration. *Pediatrics* 36: 236 1965.
- 15 Secret, R., Joffe J, Mieses P., Schatzers J & Dorval N. Un cas mortel d'intolérance héréditaire au fructose. *Pediatrics* 17: 875 1962.
- 16 Seng M, Sørensen Johansen J, Veilun J E & Gysing L R. Dietary treatment of tyrosinosis. *Acta Paediatr Scand* 57: 409 1968.
- 17 Wierman A. Studies of the activation in vitro of glucosyltransferase. *Biochim Biophys Acta* 191: 297 1969.
- 18 Wolf H P. 1 Phosphofructosaldolase. In H U Bergmeyer (ed.) *Methods of enzymatic analysis*. Academic Press, New York 1965 p 732.
- 19 Wong P W K., O'Flynn M O & Lowrye T. Micromethods for measuring phenylalanine and tyrosine in serum. *Chin Chem* 10: 1058 1964.

Submitted Sept. 2 1969

Accept of Oct 29 1969

(R. L.) Dept. of Paediatrics  
Rikshospitalet  
Oslo  
Norway

Key words: Fructosemia, acute tyrosinosis, amino acid, laboratory error of metabolism, pathological and toxicological findings.

## BILIRUBIN EXCRETION IN NEWBORN HUMAN INFANTS

## I Unconjugated Bilirubin as a Possible Trigger for Bilirubin Conjugation

ARNE F. BAKKEN

From the Paediatric Research Institute and Department of Pediatrics, University of Oslo Rikshospitalet Oslo, Norway

Jaundice is observed in 30-80 per cent of all newborn infants (6, 12, 21) and the underlying hyperbilirubinemia reaches a maximum from the third to the sixth day in normal full term infants (2, 22).

No difference can be observed between the mature and premature infants in the amount of bilirubin present at birth (2) but prematures reach a much higher level of bilirubin than mature infants (4, 13).

Bilirubin is unconjugated (UB) in cord blood both in normal fullterm and premature infants (5) because the enzyme bilirubin UDP glucuronyl transferase (BGT) (EC 2.4.1.17) is inactive at birth (17).

The first appearance of conjugated bilirubin (CB) in serum and thereby also of an active BGT has so far not been studied in detail. The hypothesis that the hyperbilirubinemia of premature infants should be due to a later than normal activation of BGT (16) has neither been based on studies of the conjugating enzyme nor on the appearance of CB in the blood. We therefore decided to study more closely the activation of bilirubin excretion in the newborn.

This investigation also showed that the first appearance of CB in all types of hyperbilirubinemia so far studied is preceded by an accumulation of UB.

This work was supported by grant no. E 303-8 from the Norwegian Research Council for Science and the Humanities.

## MATERIALS AND METHODS

*Fullterm and premature infants*

Ten fullterm and twentyfive premature infants were studied. All infants received 1 mg vitamin K<sub>1</sub> (Meadon) intramuscularly immediately after birth.

The fullterm infants were all born after normal pregnancies and had birthweights varying from 2680 to 4210 g. Blood samples were taken from the heel every day for the first eight days of life.

The premature infants had birthweights varying from 1140 to 2500 g, none of them being small for date. Two of these infants (birthweights 1140 and 1190 g respectively) died during the first week of life.

Infants with Rh or ABO incompatibility were excluded from this material.

*Infants with erythroblastosis foetalis*

This group consists of patients with erythroblastosis foetalis due to Rh immunization. The anti D titres were determined in the mothers during pregnancy by the indirect Coombs technique (The Norwegian National Institute of Public Health).

(a) Conjugated and unconjugated bilirubin were measured in heel blood samples from 25 patients which according to the maximum anti D titer found in their mothers during pregnancy were divided into the following three groups:

Group I	Max titer	0 - 128	7 cases
Group II	Max titer	256 - 512	8 cases
Group III	Max titer	1024 - 2048	10 cases

(b) Total bilirubin (TB) only was measured in 93 infants shortly after birth and they were divided according to the maximum anti D titer in their mothers during pregnancy into the following six groups:

Group 1	Max titer	0 - 128	21 cases
Group 2	Max titer	128	8 cases
Group 3	Max titer	256	16 cases
Group 4	Max titer	512	16 cases
Group 5	Max titer	1024	22 cases
Group 6	Max titer	2048	20 cases

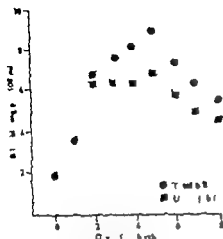


Fig 1 Total bilirubin and unconjugated bilirubin in serum from full-term infants in the first week of life. Each value is the mean of 10 sera

Three of the twenty infants in group 6 died from hydrops foetalis shortly after birth

**Newborn infants delivered from jaundiced mothers**  
Five mothers all of them jaundiced during the last week of pregnancy were examined together with their newborn infants. Three of the mothers (A, B, E, R, P, A, W) had acute hepatitis, one (A, U) suffered from an inherited type of icterus (25) and one mother (O, A) suffered from jaundice of unknown cause. Samples of blood were taken from the mothers by venipuncture and from the babies from the heel shortly after birth.

#### Exchange transfusion

Exchange transfusions were performed according to the rules described by Augusten (2, 24).

#### Bilirubin determinations

(a) Total bilirubin was determined according to Jendrasch & Gr6f (15) modified by Fog (8).

(b) Unconjugated and conjugated bilirubin were determined according to the direct spectrophotometric method described by Fog & Bakken (10).

### RESULTS

The amounts of total and unconjugated bilirubin found in normal and premature infants are shown in Fig 1. The difference between the two curves gives the amount of CB. In the full-term infants at birth TB varied from 1.1 to 2.2 mg with a mean of 1.7 mg per 100 ml. The maximum TB concentration was reached

on the fourth to sixth day with a mean of 8.6 mg per 100 ml on the fifth day.

CB was not demonstrable at birth or on the first day of life but it appeared about 48 hours after birth then varying from 0.0 to 0.6 mg per 100 ml. The maximum value of CB was reached on the third to sixth day after birth with 2.0 mg per 100 ml.

In premature infants (Fig 2) the mean value of TB at birth was 2.0 mg per 100 ml. The maximum was reached five to six days after birth with values from 11 to 19 mg per 100 ml. CB usually appeared on the second to third day while the appearance of CB in two cases was delayed until the fourth day after birth. CB increased to a maximum of 2.5 to 4.0 mg per 100 ml the third to sixth day.

The mean values of CB in the different groups suffering from erythroblastosis foetalis are shown in Fig 3. In the heaviest immunized group all the infants had CB in their sera at birth. In the moderately immunized group all had CB in their sera on the second day while four had CB even on the first day after birth. None of the least immunized infants were able to conjugate bilirubin on the first day of life. Four of them showed this ability after two days and all of them three days after birth. Maximum concentrations of CB were reached in these groups on the third, fourth and seventh day respectively. The time of exchange trans-

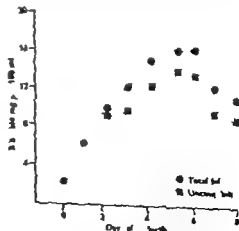


Fig 2 Total bilirubin and unconjugated bilirubin in serum from premature infants in the first week of life. Each value is the mean of 23 sera.



## BILIRUBIN EXCRETION IN NEWBORN HUMAN INFANTS

### *1 Unconjugated Bilirubin as a Possible Trigger for Bilirubin Conjugation*

ARNF BAKKEN

*From the Paediatric Research Institute and Department of Pediatrics University of Oslo Rikshospitalet Oslo Norway*

Jaundice is observed in 30-80 per cent of all newborn infants (6, 12, 21) and the underlying hyperbilirubinaemia reaches a maximum from the third to the sixth day in normal full term infants (2, 22).

No difference can be observed between the mature and premature infants in the amount of bilirubin present at birth (2) but prematures reach a much higher level of bilirubin than mature infants (4, 13).

Bilirubin is unconjugated (UB) in cord blood both in normal fullterm and premature infants (5) because the enzyme bilirubin UDP glucuronyl transferase (BGT) (EC 2.4.1.17) is inactive at birth (17).

The first appearance of conjugated bilirubin (CB) in serum and thereby also of an active BGT has so far not been studied in detail. The hypothesis that the hyperbilirubinaemia of premature infants should be due to a later than normal activation of BGT (16) has neither been based on studies of the conjugating enzyme nor on the appearance of CB in the blood. We therefore decided to study more closely the activation of bilirubin excretion in the newborn.

This investigation also showed that the first appearance of CB in all types of hyperbilirubinaemia so far studied, is preceded by an accumulation of UB.

This work was supported by grant no. II 303-8 from the Norwegian Research Council for Science and the Humanities.

## MATERIALS AND METHODS

### *Fullterm and premature infants*

Ten fullterm and twentyfive premature infants were studied. All infants received 1 mg vitamin K (menadiol) intramuscularly immediately after birth.

The fullterm infants were all born after normal pregnancies and had birthweights varying from 2680 to 4210 g. Blood samples were taken from the heel every day for the first eight days of life.

The premature infants had birthweights varying from 1140 to 2500 g, none of them being small for date. Two of these infants (birthweights 1140 and 1190 g respectively) died during the first week of life.

Infants with Rh or ABO incompatibility were excluded from this material.

### *Infants with erythroblastosis foetalis*

This group consists of patients with erythroblastosis foetalis due to Rh immunization. The anti D titers were determined in the mothers during pregnancy by the indirect Coombs technique (The Norwegian National Institute of Public Health).

(a) Conjugated and unconjugated bilirubin were measured in heel blood samples from 25 patients which according to the maximum anti D titer found in their mothers during pregnancy were divided into the following three groups:

Group I	Max titer	0 - 128	7 cases
Group II	Max titer	256 - 512	8 cases
Group III	Max titer	1024 - 2048	10 cases

(b) Total bilirubin (TB) only was measured in 95 infants shortly after birth and they were divided according to the maximum anti D titer in their mothers during pregnancy into the following six groups:

Group 1	Max titer	0 - 128	21 cases
Group 2	Max titer	128	8 cases
Group 3	Max titer	256	16 cases
Group 4	Max titer	512	16 cases
Group 5	Max titer	1024	22 cases
Group 6	Max titer	2048	20 cases

(7-16) Hyperbilirubemia due to UB might therefore be a reasonable trigger for the conjugating process. CB is found approximately 48 hours after birth in normal and premature infants. It is found even during foetal life and at birth in cases of erythroblastosis foetalis from heavily immunized mothers (Fig. 3) (1, 8, 9, 11). It has never been shown that CB has appeared for the first time *in vivo* without a preceding hyperbilirubemia due to UB. As shown for the erythroblastic infants (Figs 3 and 4) and infants born of icteric mothers (Table 1) a prenatal hyperbilirubemia might be as useful a trigger for BGT as a postnatal hyperbilirubemia for full-term and premature infants.

The prenatal activation of BGT in the heavily test immunized infant may be explained by a trigger effect upon BGT from the high level of UB in these foetuses. The moderately immunized group did not have CB at birth but these infants will have their bilirubin conjugating capacity earlier after birth than the least immunized group.

The findings in the moderately immunized group indicate that the concentration of bilirubin necessary to trigger BGT has been present prenatally for a too short period of time to finish the activation process. It seems therefore as if activation of BGT depends on the presence of a certain quantity of UB for a certain period of time.

The hyperbilirubemia in premature infants must be due to other factors than later *in vivo* normal activation of BGT (Figs 1 and 2). In erythroblastic infants the extreme hyperbilirubemia is partly due to a degradation of a greater than normal quantity of hemoglobin and partly due to the fact that CB cannot pass the placenta (19, 20).

The theory of UB being the trigger for BGT is further supported by the finding of CB in the infants whose mothers were jaundiced during the last week(s) of pregnancy (Table 1). All the five infants had CB in their sera at birth and all the mothers had UB in their sera at a level above 1.8 mg per 100 ml. According to

what has been found in monkeys (19) 1.5 mg UB per 100 ml gives a flux of bilirubin from the mother to the foetus. The foetuses from these icteric pregnant women have therefore been loaded with bilirubin before delivery.

All the infants described above got their first active BGT after a period of hyperbilirubemia *in utero* or after birth. UB may then be the trigger substance for BGT even if the activating process is influenced by other substrates or hormones as phenobarbital (23) or oestrogens (14).

These results obtained by clinical studies and the hypothetical conclusions drawn are supported by earlier studies by rats where UB has been found to be a trigger substance for bilirubin conjugation (3).

## SUMMARY

Studies of mature and premature infants in faeces suffering from erythroblastosis foetalis and infants whose mothers were jaundiced during the last week(s) of pregnancy show that the time of activation of bilirubin UDP (glucuronyl) transferase may be related to an increase of unconjugated bilirubin in the foetus or in the newborn baby. The enzyme may be activated even before birth if the load of unconjugated bilirubin is sufficiently great.

## REFERENCES

1. Rakken A. P. & Fog J. Bilirubin conjugation in the newborn. *Lancet* 1 1280 1967.
2. Rakken A. P. *Bilirubinmetabolismet skifter hos nyfødte*. Thesis Oslo 1966.
3. — Effects of unconjugated bilirubin on bilirubin (UDP-glucuronyl) transferase activity in the liver of newborn rats. *Fedat Res* 3 205 1969.
4. Billing B. H., Cole P. G. & Løthe G. H. Increased plasma bilirubin in newborn infants in relation to birthweight. *Br J Med* 1 11 1 63 1954.
5. Brown A. K. & Zuckler W. W. Studies on hyperbilirubemia. I. Hyperbilirubemia in the newborn unrelated to immunization. *Amer J Dis Child* 93 263 1957.
6. Davidson, L. T., Merritt L. L. & Welch A. L. Hyperbilirubemia of the newborn. *Amer J Dis Child* 61 938 1941.
7. Davidson, M. J. R. Changes in glucose-6-phos-

fusione were of no particular importance in determining the maxima (2)

The relationship between TB in the newborn shortly after birth and the maximum anti D titer in the mother during pregnancy is shown in Fig 4. The least immunized infants had a mean concentration of TB of 3 mg per 100 ml at birth. This level increases with increasing titer to about 6.5 mg per 100 ml in the heaviest immunized infants. The mean serum bilirubin in the heaviest immunized group was found to be about 8 mg per 100 ml if the infants which died from hydrops foetalis were excluded.

The levels of bilirubin in the jaundiced mothers and their babies are shown in Table 1. All five infants had CB varying from 0.2 to 3.5 mg per 100 ml in their sera at birth.

### DISCUSSION

The observations done in this work are based on the measurement of UB and CB in serum. The method used (10) determines the UB by direct spectrophotometry. The difference between the total bilirubin and UB is CB. This pool of CB consists largely of bilirubin glucuronide, however other bilirubin conjugates may also be present. The method has a methodological error less than 3% (2).

The appearance of CB at the same time after birth in mature and premature infants (Figs 1

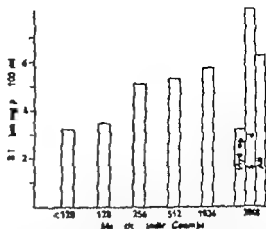


Fig 4 Total bilirubin at birth in different groups of erythroblastosis foetalis. The infants are grouped according to the max anti D titer found in their mothers during pregnancy.

and 2) seems to indicate that bilirubin conjugation has been started by something connected with delivery.

Many enzymes which are inactive during foetal life are activated shortly after birth to take care of intermediate metabolites which during pregnancy have passed the placenta to be handled by the mother. These metabolites including bilirubin accumulate when the umbilical cord is clamped. It is generally accepted that an enzyme may be activated by an increase in its own substrate. This has been shown to be valid in the newborn period too.

Table 1 Total bilirubin and conjugated bilirubin in five mothers and their newborn babies at delivery.

All the mothers were jaundiced during the last weeks of pregnancy.

Patient		Total bilirubin (mg/100 ml)	Conjugated bilirubin (mg/100 ml)
A E	mother	5.8	1.3
	child	5.3	2.6
E R P	mother	9.1	5.4
	child	10.0	2.7
A K W	mother	7.5	3.7
	child	2.8	0.2
M A	mother	2.1	0.2
	child	3.4	0.9
A U	mother	6.5	2.3
	child	6.8	3.5

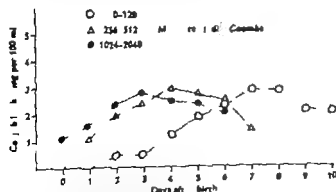


Fig 5 Conjugated bilirubin in sera from different groups of erythroblastosis foetalis during the first days of life. The infants are grouped according to the max anti D titer found in their mothers during pregnancy.

## BILIRUBIN EXCRETION IN NEWBORN INFANTS

## II Conjugated Bilirubin as a possible Trigger for Bilirubin Excretion

ARNE F. BAKKEN

From the Paediatric Research Institute and Department of Paediatrics Rikshospitalet  
University of Oslo Oslo Norway

To be excretable unconjugated bilirubin (UB) has to be coupled to glucuronic acid by the enzyme bilirubin UDP glucuronyl transferase (BGT) (EC 2.4.1.17). This conjugated bilirubin (CB) is excreted from the liver cell into the bile by an active process (9-12). Such an active process is probably also present in the kidneys.

The activation time of BGT is an important factor in the hyperbilirubinaemia found in normal newborn babies (13). The liver bilirubin excretion mechanism has been found partly to depend on hormones (6).

The amount of CB within the liver cell may decrease not only because CB is excreted into the bile but also if CB is deconjugated by the enzyme  $\beta$  glucuronidase (EC 3.2.1.31).

It has been found that UB functions as a trigger for BGT in the newborn (1,2). In this paper it is shown how CB may trigger its own excretion in normal fullterm infants, in premature infants and in infants suffering from erythroblastosis foetalis.

Increasing amounts of CB are supposed to activate its own excretion in a similar way as UB activates BGT. Both these functions have to be activated before the bilirubin is able to escape from the body through the bile or urine.

## METHODS AND MATERIALS

Total bilirubin was determined according to Jendrasak & Grod (8) modified by Fog (4). UB and CB were determined by direct spectrophotometry of diluted sera (5). All the blood samples were taken from the heel.

Bilirubin in the urine was determined by a qualitative diazo-colour reaction (Ictotest Ames Co.) which is positive when bilirubin in the urine exceeds 0.05 mg per 100 ml.

## Full term infants

Ten normal full term infants from normal pregnancies and deliveries were studied during eight days after birth. Serum CB was determined if possible each day and samples of urine were collected and analyzed simultaneously with the blood.

## Premature infants

Twentyfive premature infants were studied (birth weights 1140-2400 g). Infants with Rh or ABO incompatibilities were excluded. Twentythree infants survived the first week of life. Samples of blood and urine were collected simultaneously if possible.

## Cases of erythroblastosis foetalis

The twentyfive infants suffering from erythroblastosis foetalis were grouped according to the maximal anti D titres found in their mothers during pregnancy. (The titres determined by the indirect Coombs test by the Norwegian National Institute of Public Health.)

Group I	Max anti D-titer	0-128	7 cases
Group II	Max anti D-titer	256-512	8 cases
Group III	Max anti D-titer	1024-7048	10 cases

## RESULTS

## Fullterm infants

In normal fullterm infants CB first appeared in serum at 48 hours after birth at which time

This work was supported by grant No. E 303-8 from the Norwegian Research Council for Science and the Humanities.

- phatase activity in liver and kidney at birth *Nature* 191 72 1961
- 8 Fog J Determination of bilirubin in serum as alkaline azobilirubin. *Scand J Lab Clin Invest* 10 241 1958
  - 9 — Serumfarge og bilirubin i skleriske sera Thesis Oslo 1960
  - 10 Fog J & Bakken A F Conjugated and un conjugated bilirubin determined in icteric sera by direct spectrophotometry *Scand J Lab Clin Invest* 20 1967
  - 11 Harris L E Farrel F J Shorter R G Banner E A & Matheson D R Conjugated serum bilirubin in erythroblastosis foetalis. An analysis of 38 cases *Proc Staff Meet Mayo Clin* 37 574 1962
  - 12 Hirsch A Die physiologische Ikterus bereit schaft des Neugeborenen *Z Kinderheilk* 9 196 1913
  - 13 Hua D Y Y Allem F H Diamond A K & Gellis S S Serum bilirubin levels in the new born infants *J Pediatr* 42 277 1953
  - 14 Hua D Y Y Riabow S & Dowben E M Inhibition of glucuronyl transferase by steroid hormones *Arch Biochem Biophys* 103 181 1963
  - 15 Jendrassik L & Gróf P Vereinfachte photometrische Methoden zur Bestimmung des Serum bilirubins *Biochem Z* 297 81 1938
  - 16 Jirsa J Jirsa M & Janovsky M Importance of the quantitative determination of direct and indirect bilirubin in hemolytic disease of the new born *Acta Paediat Scand* 47 179 1958
  - 17 Lathe G H & Walker M The synthesis of bilirubin glucuronide in animal and human liver *Biochem J* 70 705 1958
  - 18 Nemeth A M Initiation of enzyme formation by birth *Ann N Y Acad Sc* 111 199 1963
  - 19 Schenker S Bashore R A & Smith F B Bilirubin disposition in foetal monkeys *in* J A Bouchier & B H Billing (eds) *Bilirubin metabolism* Blackwell, Oxford and Edinburgh 196 199
  - 20 Schmid R Bakinham S Wendells G H Hammaker L Bilirubin metabolism in the fetus *Nature* 183 1823 1959
  - 21 Waugh T R Mercant hF T G & Mangha G B Blood studies on newborn direct and total bilirubin Determination over 9-day period as special reference to icterus neonatorum *Amer Med Sci* 199 9 1940
  - 22 With T K *Biology of bile pigments* A Fra Hansen Copenhagen 1954 523
  - 23 Yaffe S J Matsugawa T & Bahab T E Enhancement of glucuronide conjugating capacity in hyperbilirubinemic infants due to apparent enzyme induction by phenobarbital *Ann Exp Med* 275 1461 1966
  - 24 Aagenæs Ø Behandling av erythroblastosis foetalis *T Norsk Lægeforen* 87 1581 1967
  - 25 Aagenæs Ø Van der Hagen C B & Ertter R Hereditary recurrent intrahepatic cholestasis from birth *Arch Dis Child* 43 646 1968

Submitted June 25 1969

Accepted Oct 16 1969

Pædiatrisch Research Institute  
Barneklmukken  
Rikshospitalet  
Oslo  
Norway

Key words Bilirubin excretion erythroblastosis foetalis newborn infants enzyme activation

## BILIRUBIN EXCRETION IN NEWBORN INFANTS

### II Conjugated Bilirubin as a possible Trigger for Bilirubin Excretion

ARNE P. BAKKEN

From the Paediatric Research Institute and Department of Paediatrics, Rikshospitalet  
University of Oslo, Oslo, Norway

To be excretable unconjugated bilirubin (UB) has to be coupled to glucuronic acid by the enzyme bilirubin UDP glucuronyl transferase (BGT) (EC 2.4.1.17). This conjugated bilirubin (CB) is excreted from the liver cell into the bile by an active process (9, 12). Such an active process is probably also present in the kidneys.

The activation time of BE is an important factor in the hyperbilirubinaemia found in normal newborn babies (13). The liver bilirubin excretion mechanism has been found partly to depend on hormones (6).

The amount of CB within the liver cell may decrease not only because CB is excreted into the bile but also if CB is deconjugated by the enzyme  $\beta$  glucuronidase (EC 3.2.1.31).

It has been found that UB functions as a trigger for BGT in the newborn (1, 2). In this paper it is shown how CB may trigger its own excretion in normal fullterm infants, in premature infants and in infants suffering from erythroblastosis foetalis.

Increasing amounts of CB are supposed to activate its own excretion in a similar way as UB activates BGT. Both these functions have to be activated before the bilirubin is able to escape from the body through the bile or urine.

This work was supported by grant no. E 309-S from the Norwegian Research Council for Science and the Humanities.

### METHODS AND MATERIALS

Total bilirubin was determined according to Jendrisak & Grof (8) modified by Fog (4). UB and CB were determined by direct spectrophotometry of diluted sera (5). All the blood samples were taken from the heel.

Bilirubin in the urine was determined by a qualitative diazo-colour reaction (Picotest, Ames Co.) which is positive when bilirubin in the urine exceeds 0.05 mg per 100 ml.

#### Fullterm infants

Ten normal fullterm infants from normal pregnancies and deliveries were studied during eight days after birth. Serum CB was determined if possible at 1 day and samples of urine were collected and analyzed simultaneously with the blood.

#### Premature infants

Twenty-five premature infants were studied (birth weights 1140-2500 g). Infants with Rh or ABO incompatibilities were excluded. Twenty-three infants survived the first week of life. Samples of blood and urine were collected simultaneously if possible.

#### Cases of erythroblastosis foetalis

The twenty-five infants suffering from erythroblastosis foetalis were grouped according to the maximal anti-D titer found in their mothers during pregnancy (The titer determined by the indirect Coombs test by the Norwegian National Institute of Public Health).

Group I	Max. anti-D-titer	0-128	7 cases
Group II	Max. anti-D-titer	256-512	8 cases
Group III	Max. anti-D-titer	1024-2048	10 cases

### RESULTS

#### Fullterm infants

In normal fullterm infants CB first appeared in serum at 48 hours after birth at which time

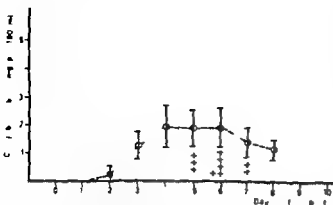


Fig 1 Conjugated bilirubin in the serum of ten normal fullterm infants (Means  $\pm$  SD). The appearance of bilirubin in the urine in the single case is indicated by +

the eight infants had from 0.1 to 0.5 mg of CB per 100 ml (Fig 1). All the infants had CB in their sera 72 hours after birth and it increased to a maximum varying from 1.8 to 3.0 mg per 100 ml. The amount then decreased to 0.1 to 1.8 mg per 100 ml eight days after birth. The highest mean values 1.9 mg per 100 ml remained unchanged from the fourth to the sixth day.

Bilirubin was detected in the urine for the first time from the fifth to the seventh day of life. Early appearance of bilirubin in the urine corresponded to an early maximum of CB in the serum.

#### Premature infants

None of the premature infants had CB in their sera until about 48 hours after birth (Fig 2).

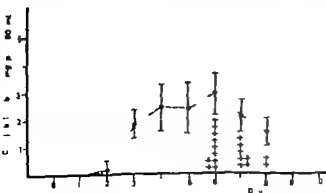


Fig 2 Conjugated bilirubin in the serum of twenty-five premature infants (Means  $\pm$  SD). The appearance of bilirubin in the urine in the single case is indicated by +

The mean values of CB in the 25 premature infants were 0.2 mg per 100 ml on the second to third day of life, varying from 0.1 to 1.0 mg per 100 ml. Maximum values of CB were reached on the third to seventh day of life with values from 2.5 to 6.0 mg per 100 ml. The highest mean value of 3.1 mg per 100 ml was reached on the sixth day after birth. The concentration of CB declined rapidly to about 1.5 mg per 100 ml on the eighth day of life.

As for the fullterm infants, bilirubin was detectable in the urine shortly after the maximum value of CB in the serum was reached. Ten of the premature infants had bilirubinuria for the first time on the sixth day of life, seven infants on the seventh day of life, and two infants on the eighth day of life.

#### Cases of erythroblastosis foetalis

The results in cases of erythroblastosis foetalis are presented in Table 1.

The maximum amount of CB in the serum was reached on the fourth to seventh second to fifth and first to fourth day in the groups I, II and III respectively. The maximum values varied from 2.8 to 5.1 mg per 100 ml in group I, 2.5 to 4.4 mg per 100 ml in group II, and 2.5 to 4.5 mg per 100 ml in group III.

While bilirubin was detectable in the urine for the first time on the sixth to ninth day in group I, it was found on the third to sixth day in group II and on the second to fifth day in group III.

#### DISCUSSION

The accumulation of CB shown in Figs 1 and 2 and in Table 1 indicates that the excretory function for this conjugate must be defective or immature at birth. It therefore seems as if a factor different from BGT has to be activated before bilirubin can be eliminated from the body. This factor becomes active at least from the fifth to the seventh day in mature and premature infants because the amount of CB in the blood from this time decreases and bilirubin from this time also can be detected in the

Table 1 Mean values of conjugated bilirubin in different groups of erythroblastosis foetalis

The first appearance of bilirubin in the urine in each case is indicated by +

Group	Max anti D titer in mother <sup>a</sup>	Days after birth										
		0	1	2	3	4	5	6	7	8	9	10
		(mg per 100 ml)										
I	0-178	0	0	0.4	0.8	1.1	1.8	2.2	2.8	2.8	2.1	2.0
II	256-512	0	1.0	1.8	2.3	2.8	2.6	2.4	1.4		++	++
					++	++	++	+				
III	1024-7048	1.1	1.5	2.3	2.7	2.4	2.3	2.0				
				++	++	++	+					
				++		+						

<sup>a</sup>Anti D titer determined by the indirect Coombs test

urine. It has been shown earlier that CB appears after birth as a possible response to an accumulation of UB and that hyperbilirubinemia for 24-28 hours was needed to activate BGT (2). It is reasonable to suppose then that accumulation of CB may act as a trigger for its own excretion in the same way as UB may trigger BGT.

Table 1 shows that the appearance of bilirubin in the urine is related both to the amount of CB present in the blood and to the duration of the increase of CB. Infants in group III for instance where the mothers had anti D titers exceeding 1024 must have had an increase of UB and even CB before birth. Some of these infants started to excrete bilirubin as early as the second day after birth in contrast to the fifth day in normal mature infants.

The excretion of CB seems to be an adaptive process in the same way as BGT is an adaptive enzyme. From the figures and Table 1 it can be anticipated that a quantity of approximately 1 mg must be present in 100 ml serum for nearly three days before the excretion of CB is really effective.

It is unknown if the liver starts to excrete CB at the same time as the kidneys. However even if the liver should begin to excrete CB somewhat earlier than the kidneys the time difference can not be greater than a few hours since no decrease in serum CB is registered until bilirubin is found in the urine.

The rate of disappearance of UB from the blood depends therefore not only on BGT as proposed by Matsuda *et al.* (10) but also on the excretion capacity for CB. The importance of the enzyme  $\beta$  glucuronidase in decreasing the concentration of CB is not yet clear.  $\beta$  glucuronidase is present in very small amounts at birth (7) and evidence is accumulating that the deconjugating capacity of this enzyme is adaptive and therefore may be triggered by an accumulation of CB (3, 11).

## SUMMARY

Studies of normal infants, premature infants and infants suffering from erythroblastosis foetalis due to Rh immunization show that conjugated bilirubin (CB) may be able to trigger its own excretion mechanism. This excretory function consists of two factors: a liver cell excretion factor and a kidney excretion factor. The role of the deconjugating enzyme  $\beta$  glucuronidase is still not clear as far as the bilirubin metabolism in the newborn is concerned.

## REFERENCES

1. Bakken A. F. Effects of unconjugated bilirubin on bilirubin UDP-glucosyl transferase activity in the liver of newborn rats. *Pediatr Res* 3: 205, 1969.
- Bilirubin excretion in newborn infants. I. Unconjugated bilirubin as a possible trigger for bilirubin conjugation. *Acta Paediatr Scand* 59: 148, 1970.



- 3 Desmet J, Bullens A M & Heirwegh K P M. Histochemistry of bilirubin and conjugates in J A M Bouchier & B H Billing (eds) *Bilirubin metabolism* Blackwell Sc Publ Oxford & Edinburgh 1967
- 4 Fog J. Determination of bilirubin as alkaline azobilirubin. *Scand J Lab Clin Invest* 10: 241 1958
- 5 Fog J & Bakken A F. Conjugated and unconjugated bilirubin determined in icteric sera by direct spectrophotometry. *Scand J Lab Clin Invest* 20: 88 1967
- 6 Gartner L M. The neonatal regulation of hepatic bilirubin excretion in J A M Bouchier & B H Billing (eds) *Bilirubin metabolism* Blackwell Sc Publ Oxford & Edinburgh 1967
- 7 Heringova A, Koldovsky O & Jirsova J.  $\beta$  glucuronidase activity in the serum of infants. *Acta Paediat Scand* 55: 175 1966
- 8 Jendrassek L & Gróf P. Verein fachte Photometrische Methoden zur Bestimmung des Bilirubins. *Biochem Z* 297: 11 1938
- 9 Jirsova J, Jursa M & Janovsky M. Importance of the quantitative determination of direct and indirect bilirubin in hemolytic disease of the newborn. *Acta Paediat Scand* 47: 179 1958
- 10 Matsuda J & Shurahata T. Bilirubin metabolism in the newborn. *Lancet* 1: 938 1967
- 11 Rana S. Histochemical determination of conjugated and unconjugated bilirubin in cholestatic liver tissue in J A M Bouchier & B H Billing (eds) *Bilirubin metabolism* Blackwell Sc Publ Oxford & Edinburgh 1967
- 12 Schenker S. Disposition of bilirubin in the fetus and the newborn. *Ann NY Acad Sci* 131: 302 1963
- 13 Schenker S, Dawber N H & Schmid R. Bilirubin metabolism in the foetus. *J Clin Invest* 41: 32 1964

Submitted June 25 1969

Accepted Oct 29 1969

Paediatric Research Institute  
Rikshospitalet  
Oslo  
Norway

**Key words** Newborn infants erythroblastosis foetal bilirubin excretion enzyme activation

## ANTHROPOMETRY IN PRIVILEGED ETHIOPIAN PRESCHOOL CHILDREN

CNU Report No 33

ROLAND EKSMYR

From the Paediatric Department University Hospital Uppsala Sweden

In Ethiopia the physical growth of children in both rural and urban settlements has been studied (13 15 18 21 27). The findings of these reports give an almost unanimous picture: the weight and height curves of infants very closely correspond to western standards during the first 3-5 months of life i.e. during the period of usually sufficient breast feeding after which the growth is retarded. At the end of the infant year and during the following preschool period the children are 20-30% underweight and almost 10% shorter than their counterparts in Europe and America.

The purpose of the present investigation was to study anthropometric data of a group of preschool purely Ethiopian children living under hygienic and socio-economic conditions approximately similar to those of average children in Europe and North America.

### MATERIAL

The children were recruited in two ways: the mothers of many of them were members of an association of educated women in Addis Ababa but most of the children were recruited from kindergartens which charged expenses less. The basic selection principle was that both parents should have secondary but not necessarily completed secondary school or better. A total of 104 children were selected by this method and investigated.

Most of the fathers and one third of the mothers had University or College degrees. The fathers were employed as engineers, managers, teachers, army of-

ficers etc. and most of the mothers were working full time usually as secretaries, nurses, social workers or teachers. Their combined income could without doubt be considered outstandingly high by Ethiopian standards. To judge by the appearance of those of their houses which were visited by the author by the availability of bathrooms, private telephones, refrigerators, cars etc. the living standard could be classed as the same or almost as high as that of many of the more privileged foreigners in Addis Ababa.

From an ethnic and religious point of view the material was heterogeneous: all the main Ethiopian ethnic groups (Amhara, Tigré, Galla, Goroja) and religions (Orthodox Christians, Moslems, Catholics and Evangelical Christians) being represented.

Age distribution was obtained for all children. Thirty-five were 36 months of age or less, the youngest being 4 months and 65 were from 37 to 60 months inclusive. Fifty-one of the 100 children were boys.

Information on birth weights was obtained for 47 children, 44 of whom had been born in hospital. Thirty of these had birth weights between 3 and 4 kg, 10 below 3 kg and 7 over 4 kg. Of those whose birth weights were under 3 kg, four were prematurely born (weighing at birth 1.5, 1.5, 1.65 and 2.35 kg).

About two thirds of the mothers had started to give supplementary food when the child was 2 months old or younger. Very few continued breast feeding for more than 4 months. The supplementary food first given was always a high quality formula made in America or Europe. The diet of the older children was according to information from the parents well balanced including eggs, meat, vegetables and fruits. Many parents gave vitamin pills regularly.

Practically all children were vaccinated the most common vaccination being BCG, triple vaccine, smallpox and poliomyelitis, but some were also vaccinated against measles and yellow fever.

In the following the children of one of the above

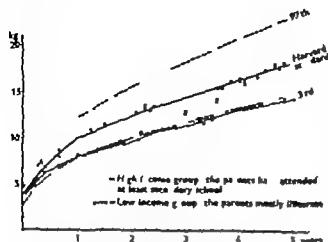


Fig. 1 Body weight of privileged Ethiopian pre-school children ( $n=100$ ). For comparison the mean weight curve for low income children ( $n=432$ ) in a typical Ethiopian region (Ijaji) (13) is given as well as the mean and the 3rd and 97th percentiles of the Harvard standard (22).

mentioned reports (13) will be referred to as "under privileged" or "low income" children. The study alluded to was carried out in a village Ijaji situated in a typical agricultural highland area 215 km west of Addis Ababa. The subjects comprised 432 children of ages 0-5 years. The methods used were the same as in the present study.

## METHODS

The nude weight of the children was recorded to the nearest 0.1 kg. For children of about and under one year of age an infant scale accurate to 0.01 kg was used and for older children a beam scale accurate to 0.1 kg. Both balances were repeatedly checked against known weights. For children shorter than about 80-90 cm an infant length board was used. For the rest of the children the standing height was measured (with steel tape attached to a wall). The length or height was recorded to the nearest full cm. For the upper arm circumference the middle of the upper left arm was measured to the nearest 0.5 cm. The triceps skinfold thickness was measured with a Harpenden caliper over the triceps muscle in the middle of the upper left arm and recorded to the nearest 0.2 cm. The chest circumference was obtained as the circumference at the nipple line on mid inspiration and the head circumference as the greatest fronto-occipital circumference both being recorded to the nearest 0.5 cm. The upper arm muscle circumference was calculated according to the formula recommended by Jelliffe (16) viz. Upper Arm Muscle Circ = Upper Arm Circ -  $\pi$  Triceps Skinfold.

The children were also examined clinically and when possible blood specimens were taken for hematological examination and amino acid screening (for methods see refs 5, 12 and 13).

## RESULTS

### A General health condition

Generally the children under study appeared clinically healthy and in as good a condition as children seen in a Well Baby Clinic in for example, Sweden. They were strikingly superior to children attending ordinary MCH Clinics in Ethiopia. Clinical signs of malnutrition, such as wasted muscles and dyspigmented hair, were never seen. Apart from one child with evidence of active rickets, no signs of Vitamin A, C or D deficiency were observed. The mean hemoglobin value for 80 children 1-5 years of age was 13.0 g/100 ml ( $SD=1.19$ ). According to the altitude adjusted WHO lower anemia limit (12) 6 children (7%) could be classified as having anemia in 2 of them the Hb was under 10.0 g/100 ml (one 8.1 and one 9.3 g%) and in 4 there was possible iron deficiency (MCHC below 27%). The microsedimentation rate was above 20 mm in about 25% of the children examined, possibly indicating present subclinical or previous infection in the other were apparently healthy subjects. Considering that the corresponding figure for Ijaji pre-school children was 80% (13) the situation of the children under study can be regarded as relatively favorable in this respect also. The amino acid ratio was 1.95  $SD=0.52$  ( $n=46$ ) supporting the parents' information that the children were receiving a well balanced protein diet.

### B Anthropometric findings

The body weight of each child under study is indicated by a black square on the Harvard growth chart of Fig. 1. It can be seen that most of the weights fell between the 3rd and 97th percentiles of the Harvard standard (22) and all except 6 were above the average weight curve of low income children in Ijaji. The weight for age distribution arranged according to Jelliffe's suggestion (16) is shown in Table 1. All children except 10 had weights for age between 80 and 120% of the standard. By comparison about half of the low income

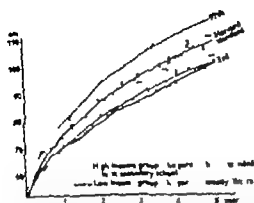


Fig 2 Heights (lengths) of privileged Ethiopian pre-school children ( $n=100$ ). For comparison the mean height curve for 'low income' children ( $n=432$ ) in a typical Ethiopian region (Ijapu) (13) as well as the mean and the 3rd and 97th percentiles of the Harvard standard (22) are also given

children 1-4 years of age in Ijapu had weights for age and 80% of the Harvard standard. The average percentage of the standard of the privileged children below 3 years of age was 102% for children between 3 and 5 years 95% and for the whole group 97% (see Table 2).

The height (length) of each child under study is indicated by a black square in a similar way as for weight in Fig 2. Most of the children had a height (length) falling within the range of the 3rd and 97th percentiles of the Harvard standard (22). The height for age ratio lay between 91 and 110% of the standard

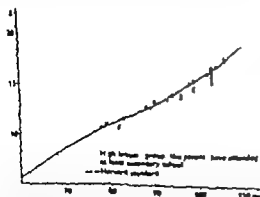


Fig 5 Weight for height of privileged Ethiopian pre-school children ( $n=100$ ). For comparison the mean of the Harvard standard (18) is also given

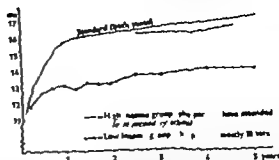


Fig 4 Mean upper arm circumference of privileged Ethiopian children 2-5 years old ( $n=83$ ). For comparison the upper arm circumference curves for 'low income' children ( $n=432$ ) in a typical Ethiopian region (Ijapu) (13) as well as the mean of the standard of reference (Jelliffe adapted from Wolanski (16)) are given

for all except 5 of the children (Table 1) the average percentage of the Harvard standard for the whole group being 99% (Table 2).

The weight for height distribution is shown in Fig 3 and Table 1 the average percentage of the Harvard standard (18) being 99%.

The upper arm circumference for age was between 91 and 110% of the standard (Jelliffe adapted from Wolanski (16)) in 70 of the 100 children (Table 1) the average percentage of the standard being 98% (Table 2). Because the number of children under two years of age was relatively small only the three age groups 25-36 months, 37-48 months and 49-60 months have been considered in Fig 4 (as well as in Figs 5 and 6). It can be seen in

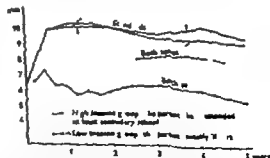


Fig 3 Mean triceps skinfold thickness of privileged Ethiopian children 2-5 years old ( $n=83$ ). For comparison the triceps skinfold curves for 'low income' children ( $n=432$ ) in a typical Ethiopian region (Ijapu) (13), as well as the means of the standards of reference (Jelliffe adapted from Henswood Tanner & Whitehouse (16)) are also given

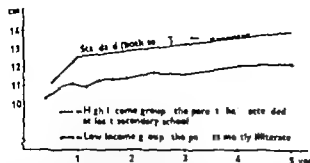


Fig 6 Mean upper arm muscle circumference of privileged Ethiopian children 2-5 years old ( $n=83$ ). For comparison the upper arm muscle circumference curve for low income children ( $n=432$ ) in a typical Ethiopian region (Ijaji) (13) as well as the mean of the standard of reference (Jelliffe calculated from the standard tables of upper arm circumference and triceps skinfold (16)) are also given

Fig 4 that the mean upper arm circumference of each of these three age groups of privileged children differs by only a few mm from the standard while the difference between the privileged and the underprivileged children (in Ijaji) is 5-8 times greater or about 2.5 cm

As seen in Fig 5 the triceps skinfold thickness of the three age groups of privileged children between 2 and 5 years was midway between that of the low income children in Ijaji and that of the standard (Jelliffe adapted from Hammond Tanner & Whitehouse (16)). The average percentage of the standard was 86% (Table 2) the distribution being wide and skewed

The upper arm muscle circumference for age

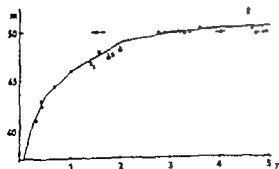


Fig 7 The head circumferences of privileged Ethiopian preschool children ( $n=78$ ). For comparison the mean of the standard of reference (Watson & Lowrey (26)) is also given

was approximately normally distributed (Table 1) with an average of 100% of the standard (Jelliffe, calculated from the standard tables of upper arm circumference and triceps skinfold (16)). As seen in Fig 6 there was a marked difference between the privileged children and the children in the typical village of Ijaji.

The chest/head ratio was initially not included in the examination form and therefore the head circumference was measured in only 78 and the chest circumference in only 46 of the 100 children. The head circumference of privileged Ethiopian preschool children (Fig 7) seems to compare well with the standard of Watson & Lowrey (26). The chest/head ratio was less than one in 3 out of 15 children 1-3 years of age and in 5 out of 31 children 3-5 years of age, i.e. in 8 (17%) of 46 children. The average weight for age of these 8 children was 88%, the average height for age 98% and the average upper arm muscle circumference 94% of the standard.

**Teeth** At the time of examination none of the 17 children of 2 years of age or less had all their deciduous teeth. Four out of 9 children of ages 25-30 months and 8 out of 9 of ages 31-36 months had all their 20 milk teeth. This was also true for all 65 children 37 months of age or older.

## DISCUSSION

To define illness we must define health. It seems obvious that a knowledge of the normal should precede the knowledge of the abnormal (14). In probably very few aspects of child health and disease is the definition of normal more controversial than in the assessment of the nutritional status, this in spite of the fact that malnutrition in its different forms and degrees is a leading pediatric problem in the developing areas of the world.

One reason for the difficulties in classifying the severity of malnutrition is the lack of universally recognized anthropometric standards (4). This is essentially due to the fact that there is still no unanimous agreement on what is the

Table 1 Some anthropometric parameters for 100 privileged preschool children in Ethiopia distributed to percentage of reference standard

% of standard group	Wt/Age	Ht/Age <sup>a</sup>	Wt/Ht	Upper arm circ/Age <sup>b</sup>	Triceps skin fold/Age <sup>c</sup>	Upper arm muscle circ/Age
121	3	0	3	0	7	1
111-100	13	1	6	5	6	14
101-110	22	31	32	33	10	41
91-100	28	64	44	46	10	37
81-90	27	4	15	14	24	6
71-80	6	0	0	1	21	1
61-70	1	0	0	1	14	0
-60	0	0	0	0	8	0
	100	100	100	100	100	100

<sup>a</sup> Standard of reference Harvard (16 22)<sup>b</sup> Standard of reference Jelliffe adapted from Wolanski (16)<sup>c</sup> Standard of reference Jelliffe adapted from Hammond, Tanner & Whitehouse (16)<sup>d</sup> Standard of reference Jelliffe calculated from the standard staples of upper arm circumference and triceps skin fold (16)

optimal development of the genetic potentiality or what is normal adaptation to environmental influences. To cite Garrow (9) Is the normal growth curve for a North American child necessarily optimum? Are children who are smaller than this necessarily malnourished?

The environmental influence on growth and physical development is well documented. That "privileged" "upper income" children are heavier and taller than underprivileged low-income children of the same race has been shown in many parts of the world e.g. India (25) West Africa (7) South Africa (17) 3) Jamaica (1) and North America (20). Also in his now classical study Greulich (10) proved that California Japanese children were taller and heavier than children in Japan. It is also common to relate the increase in mean weights and heights of children over the past

decades and centuries (in the United States (11-19) England (6) Jamaica (2) Japan (10) and other countries) to improved socio-economic conditions.

However we must take into consideration possible genetic differences. The widely accepted belief that Mongolians are shorter by heredity than Caucasians is supported by the findings of Ashcroft *et al.* (3). They found that children of similar socio-economic status in Jamaica differed in weight and height apparently due to genetic factors. Chinese children were shorter and lighter than their European and African counterparts.

The findings of the present study support the above first cited reports that children from higher socio-economic classes have a physical growth superior to that of children of the same race living under poorer conditions. The children under study had approximately the same

Table 2 Average percentage of reference standards of some anthropometric parameters for 100 privileged preschool children in Ethiopia distributed according to age groups

Age		Wt/Age	Ht/Age	Wt/Ht	Upper arm circ/Age	Triceps skin fold/Age	Upper arm muscle circ/Age
(mo)	No	(%)	(%)	(%)	(%)	(%)	(%)
4-6	25	102.1	100.3	101.4	100.0	86.3	104.6
37-60	65	94.6	97.8	97.9	97.2	85.9	100.0
4-60	100	97.3	94.7	99.0	98.2	86.1	101.6

For standards of reference see Table 1

weight, height (length) and upper arm circumference in children in industrialized countries, and they were significantly superior to their socially underprivileged counterparts in towns and rural districts of Ethiopia.

To shed further light on the possibility of a genetic factor, an attempt was made to relate the heights (lengths) of the children to the heights of their parents. However very few parents were able or willing to cooperate. The mean height of 14 fathers was 172.6 cm and that of 21 mothers 158.4 cm. These values are above the mean heights of Ethiopian adults found by the ICNND team in 1958 (15) (166.5 cm and 154.5 cm respectively) but below Caucasian standards. The number of parents, especially of fathers is too small to allow any decisive conclusions, but it would seem reasonable to interpret these findings as indicative of a secular trend towards increased growth in privileged families in Ethiopia.

The youngest children under study were relatively generally slightly heavier taller etc than the older ones. These differences do not seem to be of such an extent as to permit any detailed discussion but it may be relevant here to mention again that most of the mothers of the youngest children were members of the Association of Educated Women in Addis Ababa. These women met weekly to listen to some lecturer or to discuss between themselves topics related to Baby Care Child Health Food Economy Family Planning and so forth and they were thus a progressive elite within the total group of selected parents. It should be noted that almost all of these mothers were working full time, leaving their small children in the care of a nursemaid. It is the author's opinion that among all the various environmental factors in early childhood influencing the physical development of the child the mother's educational level occupies a central position—regardless of whether she is a housewife or professional woman working full time.

In one aspect, viz the triceps skinfold thickness the mean values of the children under study were well below the standard value for

British children (adapted by Jelliffe (16)). A possible explanation for this "anthropometric discrepancy" may be in accordance with a report from Tanzania (24) and recent findings in Ethiopia (8) that the body fat distribution of African children is different from that of Europeans.

The finding that 17% of the children in whom the chest/head ratio was measured had ratios less than one, may indicate that this sign of malnutrition should be used with some caution in nutrition surveys.

## SUMMARY

A cross sectional anthropometric survey of 100 Ethiopian children of ages 4–60 months was made. Most of the children were recruited from kindergartens charging high fees, in Addis Ababa. The basic selection principle was that both parents should have attended at least secondary school. The living standard of the children studied could be classed as equal or almost equal to that of more privileged foreigners living in Addis Ababa.

The body weight height (length) upper arm circumference triceps skinfold head circumference and chest circumference were measured. In addition the weight for height and the upper arm muscle circumference were calculated.

The results show that Ethiopian preschool children from higher socio-economic classes have a physical growth superior to that of children belonging to the same ethnic groups but living under poorer conditions. In fact, the children under study exhibited a rate of growth very close to the Western standard. This might imply that the genetic potentiality for growth (at least during the first five years of life) of Ethiopian children corresponds well to that of Caucasian children.

In one aspect viz the triceps skinfold thickness the mean values for the children under study were well below the standard of British children. This anthropometric discrepancy

may possibly be due to a difference between the body fat distribution between Ethiopian and British children

## REFERENCES

- 1 Ashcroft M T & Lovell H O Heights and weights of Jamaican children of various racial origins *Trop Geogr Med* 4 346 1964
- Changes in mean size of children in some Jamaican schools between 1951 and 1964 *J Ind Med* 14 48 1965
- 3 Ashcroft M T, Hestage P & Lovell H O Heights and weights of Jamaican schoolchildren of various ethnic groups *Ann J Phys Anthropol* 24 35 1966
- 4 Bengoa J M, Jelliffe D B & Pertierra C Years indicators for a broad assessment of the magnitude of protein-calorie malnutrition in young children in population groups *Amer J Clin Nutr* 714 1959
- 5 Bjorntorp K B, Makonnen Belew & Zaar B Biochemical study of advanced protein malnutrition in Ethiopia *Scand J Clin Lab* 20 18 1966
- 6 Clements E M B Changes in the mean stature and weight of British children over the past seventy years *Brit Med J* 11 897 1953
- 7 Coffin W R, F. Dossa I & Lea P E A Transverse survey of health and nutrition, Parks Area Division Northern Nigeria *W Afr Med J* 11 135 1962
- 8 Elseny R & Engstler G Skinfold thickness measurements in Ethiopian children To be published
- 9 Garrow J E Reply on letter by M. Lishan *Lancet* 11 279 1967
- 10 Grumbach W W A comparison of the physical growth and development of American-born and native Japanese children *Amer J Phys Anthropol* 15 439 1957
- 11 Hathaway M J Heights and weights of children and youth in the United States *Home Economics Research Reports No 3 US Dept of Agriculture Washington D C* 1957
- 12 Hofmeier Y Hematological investigations in Ethiopia *Acta Med Scand Suppl 494* 1968
- 13 Hofmeier Y & Elseny R An applied nutrition p 2 seminar in an Ethiopian community Part I (Baseline Data) Mimeographed paper Ethiopian Nutrition Institute Addis Ababa 1969
- 14 Illingworth M S *The normal child* J A Churchill Ltd London 1964 3rd edition p 7
- 15 Interdepartmental Committee on Nutrition for National Defence (ICNND) *Ethiopian Nutrition Survey* 1959
- 16 Jelliffe D B The assessment of the nutritional status of the community WHO Monograph Series No 53 1966
- 17 Kahn E & Freedman M L The physical development of a privileged group of African children *S Afr Med J* 53 934 1959
- 18 Mellbin T *et al* The nutritional status of children in five Ethiopian communities To be published
- 19 Mroczek H I Stature and weight of private school children in two successive decades *Amer J Phys Anthropol* 28 2 1941
- 20 — Stature and weight of children of the United States *Amer J Dis Child* 62 909 1941
- 21 Nebat Tefen Anthropometric studies in under privileged Ethiopian preschool children To be published
- 22 Nelson W E *Textbook of Pediatrics* Saunders Philadelphia 1964 8th edition
- 23 Phillips H J Some social and ethnic variations in the physique of South African primary school children *Arch Dis Child* 28 226 1953
- 24 Robson I R K Skinfold thickness in apparently normal African adolescents *J Trop Med Hyg* 6 209 1964
- 25 Udam P M Physical growth of children in different socio-economic groups in Bombay *Indian J Child Health* 22 497 1963
- 26 Wainman E H & Lowrey O H 1962 quoted in H B Jelliffe *The assessment of the nutritional status of the community* WHO Monograph Series No 53 p 71 1966
- 27 Woodruff C W & Hierman A Nutrition of infants and preschool children in Ethiopia *Public Health Reports* Vol 75 No 8 724 1960

Submitted Oct 11 1969

Accepted Oct 24 1969

Dept of Pediatrics  
Akademiska Sjukhuset  
Uppsala  
Sweden

Key words Ethiopian children anthropometric data



weight, height (length) and upper arm circumference as children in industrialized countries and they were significantly superior to their socially underprivileged counterparts in towns and rural districts of Ethiopia

To shed further light on the possibility of a genetic factor an attempt was made to relate the heights (lengths) of the children to the heights of their parents. However very few parents were able or willing to cooperate. The mean height of 14 fathers was 172.6 cm and that of 21 mothers 158.4 cm. These values are above the mean heights of Ethiopian adults found by the ICNND team in 1958 (15) (166.5 cm and 154.5 cm respectively) but below Caucasian standards. The number of parents especially of fathers is too small to allow any decisive conclusions but it would seem reasonable to interpret these findings as indicative of a secular trend towards increased growth in privileged families in Ethiopia.

The youngest children under study were relatively generally slightly heavier taller etc. than the older ones. These differences do not seem to be of such an extent as to permit any detailed discussion but it may be relevant here to mention again that most of the mothers of the youngest children were members of the Association of Educated Women in Addis Ababa. These women met weekly to listen to some lecturer or to discuss between themselves topics related to Baby Care, Child Health, Food Economy, Family Planning and so forth and they were thus a progressive elite within the total group of selected parents. It should be noted that almost all of these mothers were working full time leaving their small children in the care of a nursemaid. It is the author's opinion that among all the various environmental factors in early childhood influencing the physical development of the child the mother's educational level occupies a central position—regardless of whether she is a housewife or professional woman working full time.

In one aspect viz. the triceps skinfold thickness the mean values of the children under study were well below the standard value for

British children (adapted by Jelliffe (16)). A possible explanation for this "anthropometric discrepancy" may be in accordance with a report from Tanzania (24) and recent findings in Ethiopia (8) that the body fat distribution of African children is different from that of Europeans.

The finding that 17% of the children in whom the chest/head ratio was measured had ratios less than one may indicate that the sign of malnutrition should be used with some caution in nutrition surveys.

## SUMMARY

A cross sectional anthropometric survey of 100 Ethiopian children of ages 4–60 months was made. Most of the children were recruited from kindergartens charging high fees in Addis Ababa. The basic selection principle was that both parents should have attended at least secondary school. The living standard of the children studied could be classed as equal or almost equal to that of more privileged foreigners living in Addis Ababa.

The body weight, height (length), upper arm circumference, triceps skinfold, head circumference and chest circumference were measured. In addition the weight for height and the upper arm muscle circumference were calculated.

The results show that Ethiopian preschool children from higher socio-economic classes have a physical growth superior to that of children belonging to the same ethnic groups but living under poorer conditions. In fact the children under study exhibited a rate of growth very close to the Western standard. This might imply that the genetic potentiality for growth (at least during the first five years of life) of Ethiopian children corresponds well to that of Caucasian children.

In one aspect viz. the triceps skinfold thickness the mean values for the children under study were well below the standard of British children. This anthropometric discrepancy

Table 1 Cases of central nervous leukemia treated intrathecally with cytosine arabinoside

No	Initials	Age at onset of manifestation of CNS leukemia (years)	Time from onset of disease (months)	Phase of the disease in which CNS leukemia developed	Type of CNS leukemia	Treatment with methotrexate before appearance of CNS leukemia	
						Total dose mg/kg body weight	Interval after last admin. (days)
1	M R	13	4	II relapse	Meningeal	40	60
	M S	13.5	29	IV relapse	Cerebral	100	X
	M S	13.5	30	IV relapse	Cerebral	100	60
2	W J	6	11	I remission	Meningeal	14.48	160
3	T J	6	16	IV relapse	Meningeal	19.85	60
4	J B	11	6	I remission	Meningeal	7.87	X

X Administration being continued

with non-methotrexate. The blastic phases of the disease were treated with prednisone in conjunction with 4 methotrexates of methotrexate and ventricles in the lumbar bone marrow relapse routine tap of the cerebrospinal fluid revealed meningeal leukemia which at that time was not manifested by any neurologic symptoms. Methotrexate was injected intrathecally (Table 1). After the cerebrospinal fluid returned to normal, the drug was continued at longer time intervals (14-18 days) to prevent a relapse of the central nervous system.

The patient was a 11 hospitalized because of the presence of pleocytosis (56 polymorphonuclears per mm<sup>3</sup>) during control CSF examinations. The general condition of the child was good and physical examination did not reveal any abnormalities. Moderate parosmia was found at the myelogram (22%). The bone marrow relapse was treated with systemic chemotherapy. Because of possible resistance to the drugs used until then treatment with cytosine arabinoside (cytarabine<sup>1</sup>) was begun. After three intravenous in-

jections in doses of 2 mg/kg/72 hours remission in the bone marrow was obtained.

In three consecutive examinations of the CSF pleocytosis remained on a steady level and gram negative bacilli belonging to the family *Acetobacteriaceae* were isolated. Tetracycline was therefore given orally and methotrexate was repeated intrathecally. On this treatment the child's condition deteriorated: head aches, vomiting and meningeal signs appeared followed by encephalic symptoms which presented a threat to the patient's life. Pleocytosis rose to 2666/mm<sup>3</sup> (Table 1) in view of a resistance to methotrexate cytosine arabinoside was injected intrathecally in a dose of 20 mg. Two days after the injection the boy's condition improved and after three intrathecal injections of the drug pleocytosis dropped to a normal value.

During the marrow and cerebrospinal remission speech disorders appeared suddenly with subsequent convulsions<sup>2</sup> which were repeated three times. This syndrome was connected with changes in secretion of the urinary ions and marked alteration of the EEG (Table 3 Figs 1 and 2). This indicated

Supplied by Upjohn Co. Kalamazoo U.S.A.

Table 2 Methotrexate and cytosine arabinoside administered intrathecally

No	Initials	Methotrexate			Cytosine arabinoside		
		No. of injections	Total dose in mg/kg body weight	Pleocytosis after treatment	No. of injections	Total dose in mg/kg body weight	Pleocytosis after treatment
1	M S	3	0.5	10	—	—	—
	M S	3	0.3	2666	3	1.2	6
	M R	—	—	—	2	0.8	20%
2	W J	4	0.1	96	4	2.2	7
3	T J	3	0.3	103	4	3.8	54 <sup>b</sup>
4	J B	9	1.6	131	2	0.95	3

<sup>a</sup> Described in the text<sup>b</sup> After one injection of methotrexate pleocytosis returned to normal.

## CYTOSINE ARABINOSIDE ADMINISTERED INTRATHECALLY IN CEREBROMENINGEAL LEUKEMIA

B HALIKOWSKI R CYKLIS J ARMATA S GARWICZ J WYSZKOWSKI  
and M GARAPICH

*From the II nd Paediatric Clinic in the Paediatric Institute of the Medical  
Academy in Cracow (Head B Halikowski) and the EEG laboratory in  
the Outpatient Clinic of the Institute (Head J Maleski) Cracow, Poland*

Involvement of the central nervous system occurs in acute lymphoblastic leukemia in about 1/3 of cases (3-10). The basic treatment of the disease in this localization consists in the intrathecal administration of methotrexate. In addition in refractory cases of leukemia in the central nervous system X-ray therapy is employed (2, 12). A number of communications have reported the use of cytosine arabinoside and L-asparaginase in acute lymphoblastic leukemia localized in the CNS (1, 9-12). These efforts to modify the treatment of meningeal leukemia indicate that not all cases respond favorably to treatment with methotrexate and that they differ in susceptibility to therapy and in the course of their development.

Observations at our clinic incline us to believe that cases of leukemia of the central nervous system are not a homogeneous group. On the basis of the clinical symptoms, susceptibility to therapy, course, biochemical data and EEG findings we have distinguished two forms of this localization of leukemia (8).

The much more frequent meningeal form of leukemia of the central nervous system is characterized by a discrete, mild course and by a lack of symptoms of pronounced disorder of the functioning of the brain, susceptibility to intrathecal therapy and good immediate prognosis. The increased pleocytosis with or with-

out other marked signs of meningeal irritation may represent the only symptoms of CNS involvement.

The second form is marked by cerebral symptoms, less pronounced pleocytosis, fulminant course and poor immediate prognosis despite regression of the changes in the cerebrospinal fluid. This form has been called cerebral leukemia. The most striking difference between the meningeal and cerebral forms is the length of time of survival from diagnosis of meningeal leukemia to death. In 24 cases of the meningeal form the mean survival time was 12.5 months and in 3 cases of the cerebral form between several hours and 11 days. Having distinguished two forms of leukemia of the central nervous system, an attempt was made to modify therapy in the cerebral form as described below.

### CASE REPORT

In an 11-year-old boy M.S. acute lymphoblastic leukemia was diagnosed on April 5, 1965. After 5 weeks of treatment with prednisone, 6-mercaptopurine and special diet (6) bone marrow remission was obtained, followed shortly by complete remission by June 8, 1965. In the course of 30 months treatment three relapses of the disease occurred. The first relapse was localized in the testes and was treated with roentgen therapy and the next two relapses concerned the bone marrow. In the first two remissions 6-mercaptopurine was administered and in the last

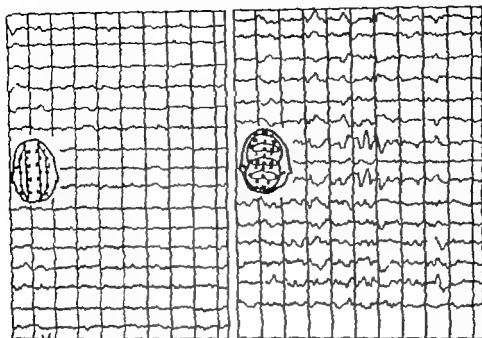


Fig 2 EEG in the child M S with the cerebral form of leukemia of the central nervous system. Series of high voltage slow waves in the deep leads

from the occipitoparietal region. In the parasagittal leads the tracing is on the boundary of normal

bral form of leukemia were quickly fatal (8). In the patient reported in this paper administration of cytosine arabinoside combined later with X ray therapy produced a remission.

Characteristic in this case was the simultaneous occurrence of clinical cerebral symptoms and a "deep" EEG alteration connected with a disturbed ionic pattern of the urine.

An analogy to the behaviour of the urine in TB encephalomyelitis described previously (4-7) pertaining to development of cerebral symptoms and occurring simultaneously with the regression of inflammatory signs in CSF should be mentioned.

In the reported case of the CNS leukemia urinary ionic changes (Table 1) were accompanied by cerebral symptoms and an alteration in the deep EEG pattern (Fig. 2). This might indicate nonspecificity of the urinary changes in cerebral disorders.

In three other cases of central nervous system leukemia refractory to treatment with methotrexate cytosine arabinoside proved effective

(Tables 1 and 2). These cases were classified by us as the meningeal form of central nervous system leukemia. Resistance to treatment in these cases manifested itself by a persistence of pleocytosis in the cerebrospinal fluid whereas in the case of cerebral leukemia described above (M S) lack of susceptibility to methotrexate was manifested by the appearance of central nervous system symptoms threatening the patient's life.

Why the population of leukemic cells in the cerebrospinal fluid became insensitive to intrathecal methotrexate is not clear. In all the reported cases the drug had been given before resistance to intrathecal methotrexate developed. The total doses of methotrexate administered before the emergence of resistance to the drug are set out in Table 1. The drug was administered as a supportive measure during remissions of either bone marrow or meningeal leukemia, and the intervals to its administration were short. Intrathecal administration of cytosine arabinoside was not attended by any

Table 3 The excretion of urinary ions and the form of CNS leukemia in boy M S

Form of CNS leukemia	Na mEq/min/1.73 m <sup>2</sup>	K mEq/min/1.73 m <sup>2</sup>	Cl mEq/min/1.73 m <sup>2</sup>	Na/K	Cl/Na
Meningeal	0.080	0.041	0.089	1.95	1.11
Cerebral	0.009	0.049	0.070	0.18	7.78
Cerebral	0.00045	0.0007	0.0005	0.64	1.11
Normal values(5)	0.165 ±0.058	0.037 ±0.017	0.188 ±0.064	5.00 ±2.04	1.26 ±0.16

that the cerebral form of CNS leukemia had developed. In view of the suspected blastic proliferation in the cerebral tissues radiotherapy of the skull was performed resulting in a regression of all neurologic symptoms.

During the third relapse of cerebromeningeal leukemia intrathecal injection of cytosine arabinoside proved ineffective and normalization of the regression of clinical symptoms was achieved by increasing the intrathecal dose of methotrexate to 0.2 mg/kg body weight. The child had two further bone marrow relapses with an enlargement of the kidneys during the last one. The boy died 35 months after the beginning of treatment.

Autopsy revealed extensive leukemic infiltrations of the internal organs mainly existing in the kidneys

generalized hemorrhagic diathesis cerebral edema and internal hydrocephalus.

## DISCUSSION

In the reported case of acute lymphoblastic leukemia, there was a pronounced tendency to extramedullary relapses of the disease, manifested by involvement of the testes, by localization in the CNS on 3 occasions and renal tumors in the terminal stage of the disease. Cerebro meningeal leukemia was detected twice during routine lumbar taps and clinical symptoms appeared later. Asymptomatic course was observed in the early phase of the meningeal form at the beginning of relapse despite the existing cerebrospinal fluid changes. On the basis of the clinical symptoms and the results of laboratory examinations the course of the CNS relapses was classified as the meningeal form. Later cerebral changes developed with disturbances in the ions of the urinary excretion and an alteration of the EEG trace.

Three previously observed cases of the cere

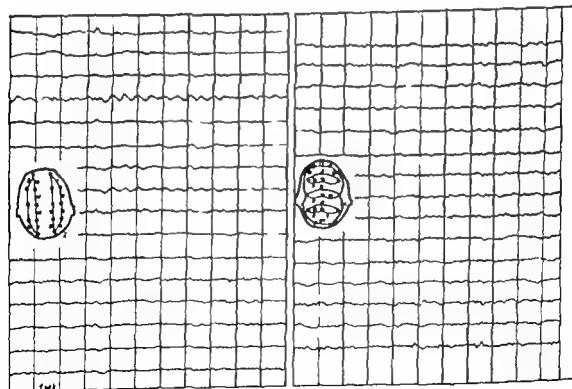


Fig 1 EEG in the child M S with the meningeal form of leukemia of the central nervous system. Numerous delta waves in the parasagittal leads from

the occipitoparietal region especially on the right side. In the deep leads the tracing is on the boundary of normal.

## LONGITUDINAL STUDIES OF INTELLECTUAL AND BEHAVIORAL DEVELOPMENT IN CHILDREN WITH CONGENITAL HEART DISEASE

LEONARD M. LINDE, BEATRICE RASOF and OLIVE JEAN DUNN

From the Departments of Pediatrics, Public Health and Preventive Medicine  
University of California at Los Angeles, Los Angeles, California, U.S.A.

This is the final paper in a series (5 of 9) which reports the results of a five year developmental study comparing the intelligence and adjustment of children with cyanotic congenital heart disease, those children with acyanotic cardiac disease and normal children. Previous papers have not dealt with changes during the five year study period.

Possible effects of intervening cardiac operation upon the development of children with congenital heart disease are of primary interest. More than 40% of the children in the cardiac group underwent operation during the course of the investigation and so we were able to compare the development of operated and non-operated children. We studied changes in physical capacity, intelligence and adjustment of the child and also changes in the mother's attitude toward her child, the extent of her anxiety and her need to protect and pamper him.

### METHODS

**Subjects**  
A total of 119 children were studied. Forty nine boys and 49 girls with congenital cardiac malformation, marked cyanosis and physical handicap (Group 1) were compared with 1 boy and 48 girls with congenital cardiac disease, handicap but without cyanosis (Group 2). Group 3 consisted of 51 normal siblings born in age to the congenitally handicapped pa-

tients while Group 2 was composed of 40 normal children chosen at random from a well baby clinic. Patients in whom the heart disease was one manifesting signs of a general syndrome which might have included mental retardation were excluded from the study.

**Assessment of intelligence and developmental status**  
Each subject was administered a developmental and/or intelligence test appropriate to his age. Children under 2 1/2 to 3 years received both the Cattell Infant Intelligence Scale and the Gesell Developmental Schedules. From age 2 1/2 to 3 the revised Stanford-Binet Scale, Form L and M were used. Forms were administered in order to maximize practice effects. Children were tested at 6 month intervals below the age of 3 and at yearly intervals above 3 years of age. Ideally children first tested at age 6 months were retested with the Gesell and Cattell at ages 1 1/2, 2 and 2 1/2 years. At age 3 Stanford-Binet Form L, and at age 4 Stanford-Binet Form M were administered. Interviews with children with cardiac disease were added to this study in order of their first appearance at the hospital test sequence age sequence and number of tests vary among children. When cardiac operation intervened, psychological tests were performed preoperatively 3 to 6 months postoperatively and then at yearly intervals until stable results were obtained or until the project was completed.

The child's behavior during the test was rated for attention, willingness, self-confidence, social confidence and physical level of activity.

Semi-structured interviews with the mother were conducted at the time of each test by the examining psychologist. Seven point rating scales were devised for maternal attitude, child adjustment and physical assessment variables which could be rated. In view of the longitudinal nature of the study the psychologists usually were aware of the patient's course including the retesting and the personal interviews whether or not operation had been performed.

Supported by United States Public Health Service Contract no. H1D-00639 and the UCLA Community Facility.

toxic symptoms. It may be concluded that cytosine arabinoside administered intrathecally extends the range of drugs used in the treatment of leukemia of the central nervous system.

### SUMMARY

Two clinical forms of CNS leukemia in children may be distinguished: the meningeal and the cerebral. The following features constitute the differences between these two forms: the clinical symptoms, the number of cells in the CSF, EEG trace, urinary findings and survival time. The main difference, however, is revealed in the response to routine therapy.

Therefore, some attempt has been made to vary the therapy. Arabinoside of cytosine was also used intrathecally in the meningeal form of CNS leukemia in cases with a small degree of sensitivity to methotrexate therapy.

### REFERENCES

1. Borron M, Jacquillat C, Weil M, Tanzer J & Bernard J. Lasparaginase. *Presse Med* 76: 2099, 1968.
2. Evans A B, D'Angio G J & Mitus A. Central nervous system complications of children with acute leukemia. An evaluation of treatment methods. *J Pediatr* 64: 94, 1964.
3. Haghbin M & Zuelzer W W. A long term study of cerebrospinal leukemia. *J Pediatr* 67: 23, 1965.
4. Halikowski B. The pathogenesis and the course of tuberculous encephalo meningitis. (In Polish) *Gaz. Jena* 22: 461, 1954.
5. — Insuffisance du tubule renal au cours de l'encephalo meningite tuberculeuse des enfants et

son importance pour le pronostic. *16 eme Congrès Ped. Lang. Franc.* Paris 1957, p. 29.

6. Halikowski B, Armata J & Garwicz S. Low protein purine free diet in treatment of acute leukemia in children: preliminary communication. *Brit Med J* 1: 549, 1966.
7. Halikowski B, Kłita S & Nawarecki H. The correlation of the Na, K, and Cl ionic contents, pH, volume and concentration ratio of the urine to the "cerebral" symptomatology in TB meningitis in children. *X Intern. Congr. Paed.* Lisbon 1962, p. 262.
8. Halikowski B, Wyszowski J, Armata J, Garwicz S, Cykalis R & Bryszak C. The "meningeal" and "cerebral" forms of leukemia of the central nervous system in children. (In Polish) *Pediatr. Pol.* 43: 145, 1968.
9. Howard J P, Aitbo V & Newton Jr W A. Cytosine arabinoside. Results of a cooperative study in acute childhood leukemia. *Cancer* 21: 341, 1968.
10. Hyman C B, Bogle J M, Brubaker Ch A & Williams K. Central nervous system involvement of leukemia in children. I. Relationship in systemic leukemia and description of clinical and laboratory manifestation. *Blood* 25: 1, 1965.
11. Kozłowska J & Rokucha Milewska R. The infiltrations of the central nervous system in the course of acute leukemia in children. (In Polish) *Pediatr. Pol.* 42: 919, 1967.
12. Najean Y, Jacquillat C, Weil M, Tanzer J & Borron M. Orientation actuelle de la chimiothérapie des leucémies aigües. *Vie Med* 4: 949, 1967.

Submitted Nov. 21, 1968

Accepted June 10, 1969

(B. H.) *Inst. Paediatric Clinic*  
Pediatric Institute  
Kraków - Prokocim  
Poland

**Key words:** Arabinoside, cytosine, CNS leukemia, assistance to therapy.

Table 2 IQ means for cardiac groups

Unadjusted means for first and last tests and means at last test adjusted for six covariates

Unadjusted means for first and last tests from patients in each test category							
Group (1)	$\lambda$ (2)	First test		Last test		Adjusted mean (7)	s.d. (8)
		Mean (3)	s.d. (4)	Mean (5)	s.d. (6)		
<i>Acyanotic</i>							
Operated 35	71	102.5	2.5	103.4	2.0	99.8	2.2
Nonoperated 35	45	105.2	3.0	109.9	1.7	105.1	2.0
<i>Cyanotic</i>							
Operated 45	31	99.4	2.7	104.8	2.1	107.1	2.5
Nonoperated 45	43	94.6	3.0	95.1	1.7	105.2	2.1

the rankings of the IQs we observed at the time of the last test cannot be explained by differences in age composition of the groups or by differences in economic level (as measured by the education of the father).

It was further found that the inclusion of incapacity as a covariate always resulted in changes in the rank orders of the adjusted means. Exclusion of incapacity always left the rankings the same as those of the unadjusted means (Table 2 Column 5). Whenever incapacity was included the ranking of the mean was as in Table 2 Column 7.

Covariance analyses in a survey are difficult to interpret. An explanation of the adjusted means in Table II might be as follows: looking separately at the acyanotic and the cyanotic groups. Differences in incapacity, age and initial IQ cannot explain more than part of the difference observed between final IQ for the 45 and 45 subgroups so that we might possibly attribute the remaining difference (107.1 to 101.1) to the operation.

Similarly, in comparing the two subgroups of the acyanotic children we see that the difference in final IQ (103.4 to 109.9) cannot be attributed to initial IQ or incapacity. The reason for this difference is not clear, but factors involved in selection for operation or of facts of operation itself may be involved.

In comparing the cyanotic children with the acyanotic one should be especially cautious. We see here that in the event that an operated

cyanotic child has initial incapacity and initial IQ equal to an unoperated acyanotic his IQ on the last test might be expected on the average to be higher than that of the acyanotic child. Since cyanotic children often have physical handicaps which give spontaneously lower developmental quotient on tests performed at a lower age, statistical adjustment raises their final IQ. In other words, the cyanotic children did relatively well on final IQ considering their age, initial IQ and their incapacity.

Initial low IQ in the cyanotic children is related at least in part to their incapacity. Therefore the increase in IQ in this group following successful operation with its attendant clinical improvement is readily predicted and explained. The lack of IQ increase in the unoperated cyanotic children is expected in a similar way. In the acyanotic children, initial handicap is less and IQ at first testing is therefore closer to the true potential of the individual.

The tendency to increase in IQ in the unoperated acyanotic children may be explained by a combination of initial condition and selection. This subgroup consists of children who entered the study with a mean incapacity slightly lower than that of the other acyanotic subgroup. Nothing untoward occurred during their period on the project to accumulate an operation; they did relatively well.

To study the changes in IQ for different diagnostic categories within the cyanotic



Table 1 IQ means for first and last tests and mean changes for normal and cardiac groups

Group (1)		<i>N</i> (2)	Mean at first test (3)	Mean at last test (4)	Difference (5)	SE of difference (6)	<i>t</i> (7)
Well baby	1	39	112.8	114.5	1.7	2.12	.80
Sibling	2	68	110.3	113.9	3.6	1.30	1.77
Acyanotic operated (S)	3S	35	102.3	103.3	.9	1.66	.4
Acyanotic non operated (S)	3S	45	105.2	109.9	4.7	2.40	1.95
Cyanotic operated	4S	33	99.9	105.2	5.3	1.89	2.79
Cyanotic non operated	4S	45	94.6	95.1	.5	1.71	.28

Differences between mean IQ's first and last test are significant using a single test level of 0.05

A complete description of methods for assessing child adjustment and maternal attitude appears in Lande et al (5). For a more detailed account of sampling procedures and the assessment of physical status refer to Lande et al (7).

#### Methods of analysis

Preliminary analyses included all data collected during the course of the investigation. Longitudinal developmental measures were graphed and studied for information from all tests. Because of (1) variations in both the number of tests per child and the length of time each child was studied and (2) the need to establish a basis for a meaningful comparison between surgical and non-surgical groups, the first and last test measures were selected for final analysis. The data were analyzed in several ways: (1) Group means of variables measured at the first and last tests were compared within each group studied (cyanotic without surgery, 4S; cyanotic with surgery, 4S; acyanotic without surgery, 3S; acyanotic with surgery, 3S; siblings of children, 2; well babies, 1). (2) A comparison among all groups of changes in the means of attitudinal and intellectual variables between the first and last tests was made. (3) Covariance analyses of IQ on the last test for surgical and non-surgical cardiac groups were made on a selected set of covariates (incapacity at the first test, incapacity at the last test, the square root of age at the first test, education of the father at the first test, and IQ measured on the first test). This analysis makes it possible to determine whether the differences observed among the groups in IQ measured on the last test might be explained by differences among any of the covariates.

### DISCUSSION

#### Intellectual development

A comparison of changes in IQ from the first to the last test within groups (Table 1) shows that all groups made some gains in mean IQ, a finding which may be related to improvement with practice. (1) The major gain was in the

cyanotic group with intervening surgery; the only statistically significant change among cardiac groups. Normal siblings of children with cardiac disease also showed gains. However, when increases in IQ for operated groups were compared with increases in IQ for non-operated groups, none of the comparisons was statistically significant.

The failure of the operated acyanotic children to improve appreciably and the almost significant gain in IQ for the acyanotic group without operation ( $t=1.95$ ) were unanticipated findings. An effort was made to understand and interpret the entire pattern of changes among the cardiac groups. For this purpose, a covariance analysis was performed. Such an analysis can help to determine if the observed changes in IQ can be explained in terms of the initial composition of the groups or in terms of other variables measured at the time of the last test.

A covariance analysis was performed with IQ on the last test as the dependent variable and with age, education of the father, IQ and incapacity at the time of the first test, and age and incapacity at the time of the last test as covariates. Various combinations of the six covariates were used in an attempt to understand the adjusted means of final IQ.

It was seen first of all that the only covariates whose inclusion or exclusion made any difference in the analysis were IQ at the first test and incapacity, either at the first or last test. Age and education of the father made no difference. This gives us some assurance that

Table 2 IQ means for cardiac groups

Unadjusted means for first and last tests and means at last test adjusted for six covariates

Group (1)	N (2)	First test		Last test		Adjusted mean (7)	SE (8)
		Mean (3)	SE (4)	Mean (5)	SE (6)		
Acyanotic							
Operated 35	34	102.5	2.5	103.4	2.0	99.8	2.2
Nonoperated 35	45	105.2	3.0	109.9	1.7	105.1	2.0
Cyanotic							
Operated 45	31	99.4	2.7	104.8	2.1	107.1	2.5
Nonoperated 45	45	94.6	3.0	95.1	1.7	101.1	2.1

the rankings of the IQs we observed at the time of the last test cannot be explained by differences in age composition of the groups or by differences in economic level (as measured by the education of the father).

It was further found that the inclusion of incapacity as a covariate always resulted in changes in the rank orders of the adjusted means. Exclusion of incapacity always left the rankings the same as those of the unadjusted means (Table 2 Column 5). Whenever incapacity was included the ranking of the means was as in Table 2 Column 7.

Covariance analyses in a survey are difficult to interpret. An explanation of the adjusted means in Table 11 might be as follows: looking separately at the acyanotic and the cyanotic groups. Differences in incapacity, age and initial IQ cannot explain more than part of the difference observed between final IQ for the 45 and 45 subgroups so that we might possibly attribute the remaining difference (107.1 to 101.1) to the operation.

Similarly in comparing the two subgroups of the acyanotic children we see that the difference in final IQ (103.4 to 109.9) cannot be attributed to initial IQ or incapacity. The reason for this difference is not clear but factors involved in selection for operation or effects of operation itself may be involved.

In comparing the cyanotic children with the acyanotic one should be especially cautious. We see here that in the event that an operated

cyanotic child has initial incapacity and initial IQ equal to an unoperated acyanotic his IQ on the last test might be expected on the average to be higher than that of the acyanotic child. Since cyanotic children often have physical handicaps which give spuriously lower developmental quotient on tests performed at a lower age, statistical adjustment raises their final IQ. In other words the cyanotic children did relatively well on final IQ considering their age, initial IQ and their incapacity.

Initial low IQ in the cyanotic children is related at least in part to their incapacity. Therefore the increase in IQ in this group following successful operation with its attendant clinical improvement is readily predicted and explained. The lack of IQ increase in the unoperated cyanotic children is expected in a similar way. In the acyanotic children initial handicap is less and IQ at first testing is therefore closer to the true potential of the individual.

The tendency to increase in IQ in the unoperated acyanotic children may be explained by a combination of initial condition and selection. This subgroup consists of children who entered the study with a mean incapacity slightly lower than that of the other acyanotic subgroup. Nothing untoward occurred during their period on the project to necessitate an operation; they did relatively well.

To study the changes in IQ for different diagnostic categories within the cyanotic

Table 1 *IQ means for first and last tests and mean changes for normal and cardiac groups*

Group (1)		N (2)	Mean at first test (3)	Mean at last test (4)	Difference (5)	S.E. of difference (6)	t (7)
Well baby	1	39	112.8	114.5	1.7	7.12	.40
Sibling	2	68	110.3	113.9	3.6	1.30	2.7*
Acyanotic operated (S)	3S	35	102.3	103.3	.9	1.66	.56
Acyanotic non operated (S)	3S	45	105.2	109.9	4.7	2.40	1.95
Cyanotic operated	4S	33	99.9	105.2	5.3	1.89	2.8*
Cyanotic non operated	4S	45	94.6	95.1	.5	1.71	.28

\* Differences between mean IQs first and last test are significant using a single test level of 0.05

A complete description of methods for assessing child adjustment and maternal attitude appears in Linde *et al* (5). For a more detailed account of sampling procedures and the assessment of physical status refer to Linde *et al* (7).

#### Methods of analysis

Preliminary analyses included all data collected during the course of the investigation. Longitudinal developmental measures were graphed and studied for information from all tests. Because of (1) variations in both the number of tests per child and the length of time each child was studied and (2) the need to establish a basis for a meaningful comparison between surgical and non surgical groups, the first and last test measures were selected for final analysis. The data were analyzed in several ways. (1) Group means of variables measured at the first and last tests were compared within each group studied (cyanotic without surgery, 4S cyanotic with surgery, 4S acyanotic without surgery, 3S acyanotic with surgery, 3S siblings of cardiacs, 2 well babies, 1). (2) A comparison among all groups of changes in the means of attitudinal and intellectual variables between the first and last tests was made. (3) Covariance analyses of IQ on the last test for surgical and non surgical cardiac groups were made on a selected set of covariates (incapacity at the first test, incapacity at the last test, the square root of age at the first test, the square root of age at the last test, education of the father at the first test and IQ measured on the first test). This analysis makes it possible to determine whether the differences observed among the groups in IQ measured on the last test might be explained by differences among any of the covariates.

## DISCUSSION

### Intellectual development

A comparison of changes in IQ from the first to the last test within groups (Table 1) shows that all groups made some gains in mean IQ, a finding which may be related to improvement with practice. (1) The major gain was in the

cyanotic group with intervening surgery, the only statistically significant change among cardiac groups. Normal siblings of children with cardiac disease also showed gains. However, when increases in IQ for operated groups were compared with increases in IQ for non operated groups, none of the comparisons was statistically significant.

The failure of the operated cyanotic children to improve appreciably and the almost significant gain in IQ for the acyanotic group without operation ( $t = 1.95$ ) were unanticipated findings. An effort was made to understand and interpret the entire pattern of changes among the cardiac groups. For this purpose, a covariance analysis was performed. Such an analysis can help to determine if the observed changes in IQ can be explained in terms of the initial composition of the groups or in terms of other variables measured at the time of the last test.

A covariance analysis was performed with IQ on the last test as the dependent variable and with age, education of the father, IQ and incapacity at the time of the first test and age and incapacity at the time of the last test as covariates. Various combinations of the six covariates were used in an attempt to understand the adjusted means of final IQ.

It was seen first of all that the only covariates whose inclusion or exclusion made any difference in the analysis were IQ at the first test and incapacity either at the first or last test. Age and education of the father made no difference. This gives us some assurance that

Table 4 Psychological adjustment variables  
Means at first and last tests and mean changes<sup>a</sup>

Means at first and last tests and mean changes							
Group (1)		N (2)	Mean at first test (3)	Mean at last test (4)	Difference (5)	% of difference (6)	t (7)
<b>(a) General adjustment</b>							
Well baby	1	37	2.84	2.81	-.03	17	-1.7
Sibling	2	61	2.88	3.13	.25	16	1.43
Acyanotic operated	35	24	3.15	3.09	-.06	20	-.30
Acyanotic nonoperated	38	46	2.70	2.98	.28	16	1.78
Cyanotic operated	48	34	3.62	2.79	-.83	23	-3.62 <sup>a</sup>
Cyanotic nonoperated	45	48	2.96	3.25	.29	16	2.13 <sup>a</sup>
<b>(b) Willingness</b>							
Well baby	1	37	2.35	.11	.24	15	-1.63
Sibling	2	67	2.36	2.21	-.15	13	-1.49
Acyanotic operated	35	38	2.34	2.33	.01	14	.35
Acyanotic nonoperated	35	51	2.37	2.41	.04	13	.30
Cyanotic operated	48	34	2.79	2.21	-.58	18	-3.23 <sup>a</sup>
Cyanotic nonoperated	45	51	2.35	2.43	.08	17	.72
<b>(c) Social confidence</b>							
Well baby	1	38	2.61	2.39	-.22	19	-1.13
Sibling	2	68	2.28	2.41	.13	12	1.10
Acyanotic operated	38	38	2.55	2.60	.05	19	.26
Acyanotic nonoperated	35	51	2.41	2.43	.02	19	.0
Cyanotic operated	48	34	3.03	2.59	-.44	20	-2.21 <sup>a</sup>
Cyanotic nonoperated	45	51	2.61	2.57	-.04	16	-.26
<b>(d) Attention</b>							
Well baby	1	38	2.66	2.47	-.19	20	-.93
Sibling	2	68	2.54	2.40	-.14	15	-.94
Acyanotic operated	38	38	2.79	2.61	-.18	20	-.91
Acyanotic nonoperated	35	51	2.55	2.53	-.02	17	-.11
Cyanotic operated	48	34	3.03	2.09	-.94	18	-5.12 <sup>a</sup>
Cyanotic nonoperated	45	51	3.06	2.71	-.35	18	-1.91

<sup>a</sup> Negative change signifies improvement.

<sup>b</sup> Mean changes from first to last test are significant using a single test level of 0.05.

changes in the combined non-operated groups (1+2+35+45) it is again clear that only the operated cyanotics make significant gains in general adjustment, attention and willingness.

Although ratings of the child's anxiety were obtained from the parent interview many mothers were unable to determine if the child was anxious or not particularly in the younger children. Consequently data do not permit any statement about this important variable.

#### Changes in physical status

The intervention of cardiac operation seemed particularly beneficial in reducing the symptoms associated with physical exercise for the cyanotic child (Incapacity Table 5). Acyanotic children though initially less incapacitated showed less but also significant improvement.

Less striking but comparable changes in directions were noted in the child's level of physical activity as manifested in the testing situation. This measure is based on the psychologist's assessment and is independent of the measure of incapacity rated by the physician. When one compares the improvement within each group separately with changes in the combined non-operated groups (1+2+35+45) it is only the cyanotic children who show significantly increased level of physical activity. The normal children and cardiac children without surgery remain substantially unchanged.

#### Change in maternal attitude

Improvement in the child was accompanied by significant changes in maternal attitudes. The mothers of both cyanotic and acyanotic chil-

Table 3 IQ means for first and last tests and mean changes for Cyanotic group

Two diagnostic categories

Group (1)	N (2)	First test		Last test		IQ change (7)
		Mean (3)	s.e. (4)	Mean (5)	s.e. (6)	
<i>Cyanotic tetralogy of Fallot (A)</i>						
Operated 4SA	21	97.9	3.5	104.0	3.5	+6.1
Nonoperated 4SA	18	93.9	5.3	99.5	4.6	+5.6
<i>Other cyanotic conditions (B)</i>						
Operated 4SB	12	103.5	3.9	107.2	5.3	+3.7
Nonoperated 4SB	27	95.1	3.7	92.1	4.1	-3.0

group the group was further subdivided into children with tetralogy of Fallot (Group 4 category A) and those with other cyanotic conditions (Group 4 category B). Table 3 shows the IQ means on the first and last tests in these diagnostic categories. It was found that among non-operated children cyanotic tetrads improved in IQ while the other diagnostic category declined in IQ. This difference in IQ changes between the two non-operated subgroups was statistically significant. The IQ changes found in the two diagnostic categories of operated cyanotic children were both positive (6.1 and 3.7). Almost no difference between operated and non-operated cyanotic children was found in diagnostic category A (tetralogy of Fallot); the difference was large between operated and non-operated children in category B (cyanotic conditions other than tetralogy of Fallot). Operated and non-operated cyanotic tetrads gained in IQ while other cyanotic children gained only when operation intervened. The difference in changes in IQ between the two diagnostic categories in the non-operated but not in the operated group is understood only when one takes selective factors and disease severity into consideration. Although age at testing, arterial oxygen saturation, hemoglobin and hematocrit were similar in all groups, the non-operated cyanotic group includes a larger number and a greater proportion of inoperable cases with an associated poor prognosis. Thus one might expect greater

differences between the two diagnostic categories in the non-surgical group and small differences in the surgical group which are more nearly equal in terms of prognosis in initial condition. There is a possibility that a decrease in IQ in the inoperable cyanotic children may be related to altered attitudes of physicians, parents or testing psychologist.

#### Emotional development

The greatest improvement in psychological adjustment and behavioral variables is found in the cyanotic group with surgery when one compares within-group changes from the first to the last test (Table 4). The reader is reminded that lower values of the variables signify better adjustment: less anxiety etc. A negative difference between the last and first measurement represents improvement. Among all the groups it is only the operated cyanotic children who show statistically significant gain in general adjustment, self-confidence, social confidence, willingness and attention. An interesting observation is that the non-operated cyanotic group showed a significant decline in general adjustment. Changes in this variable within the normal group and the two cyanotic groups remained unchanged. Intervention surgery appeared to have little influence on the degree of physical dependence shown by the child.

When one compares the improvement with each operated group separately with the

Table 6 Maternal attitude

Means at first and last tests and mean changes

Group (1)		N (2)	Mean at first test (3)	Mean at last test (4)	Difference (5)	st. of difference (6)	t (7)
(a) <i>Proleptic anxiety</i>							
Well baby	1	36	11	2.22	11	12	.93
孪生	2	61	2.02	2.13	.11	.08	1.35
Acyanotic operated	33	34	3.46	2.88	-.60	.21	-3.89 <sup>a</sup>
Acyanotic nonoperated	15	46	3.37	3.07	-.30	.13	-2.38 <sup>a</sup>
Cyanotic operated	45	33	4.15	3.24	-.90	.21	-4.33 <sup>a</sup>
Cyanotic nonoperated	45	48	4.23	4.10	-.13	.15	-.96
(b) <i>Pampering</i>							
Well baby	1	37	2.34	2.54	0	.17	0
孪生	2	61	1.96	2.21	.23	.13	1.74
Acyanotic operated	33	34	3.30	2.68	-.62	.20	-4.09 <sup>a</sup>
Acyanotic nonoperated	15	46	2.67	2.49	-.18	.16	-1.39
Cyanotic operated	45	34	3.97	2.97	-1.00	.23	-4.40 <sup>a</sup>
Cyanotic nonoperated	45	48	3.94	3.71	-.23	.18	-1.27
(c) <i>Maternal anxiety</i>							
Acyanotic operated	33	33	3.97	3.06	-.91	.20	-3.01 <sup>a</sup>
Acyanotic nonoperated	15	46	3.50	3.17	-.33	.13	-.99
Cyanotic operated	45	33	4.17	3.23	-.94	.20	-4.69 <sup>a</sup>
Cyanotic nonoperated	45	48	3.94	4.00	.06	.16	.39

<sup>a</sup> Negative change signifies improvement<sup>b</sup> Mean changes from first to last test are significant using a single test level of 0.05

## CONCLUSIONS

The effects of congenital heart disease on intellectual and emotional development have been studied. Children with cyanotic and acyanotic cardiac disease their normal siblings and children selected from a well baby clinic were evaluated longitudinally over a five year period. The effects of time and intervening cardiac operation on the children's development and in parental attitudes toward the child were measured.

Cyanotic children showed a lag in intellectual development particularly in early years related in part to physical incapacity. IQ increased in operated cyanotic children but not in the inoperable cyanotic group. Children with acyanotic congenital cardiac disease were less incapacitated initially and their original IQ was closer to their true potential therefore less change was seen with time whether or not cardiac operation intervened.

Greatest improvement in psychological adjustment and behavior was seen in the operated cyanotic children with less change

in the other groups of normal children and those with heart disease. After operation mothers of all cardiac children were less anxious about them and pampered and protected them less. The data imply a relationship between the magnitude of parental concern and the presence of cyanosis, a concern related only partly to the fact that these children were all more incapacitated.

Physical ability increased in all children after operation but more so in those who were cyanotic preoperatively. Physical status did not change in normal children and in unoperated children with heart disease.

The data indicate that operative correction of the cardiac lesion and attendant alleviation of the accompanying symptoms are associated with improvement in intellectual functioning particularly in the cyanotic group. In addition the visibility of cyanosis in particular appears to stimulate parental anxiety and its disappearance postoperatively is accompanied by the most marked decrease in parental concern.

Table 5 *Physical status*Means at first and last tests and Mean changes<sup>a</sup>

Group (1)		N (2)	Mean at first test (3)	Mean at last test (4)	Difference (5)	s.e. of difference (6)	t (7)
<i>(a) Physical level of activity</i>							
Well baby	1	37	3.62	3.67	.05	15	.32
Sibling	2	68	3.76	3.68	-.08	10	-.68
Acyanotic operated	3S	38	4.05	3.82	-.23	14	-1.61
Acyanotic nonoperated	3S	48	3.52	3.69	.17	18	1.04
Cyanotic operated	4S	32	4.34	3.91	-.43	17	-2.43 <sup>b</sup>
Cyanotic nonoperated	4S	49	3.88	4.14	.26	19	1.36
<i>(b) Incapacity</i>							
Acyanotic operated	3S	36	2.08	1.58	-.50	24	-2.05 <sup>b</sup>
Acyanotic nonoperated	3S	53	1.85	1.62	-.23	12	-1.80
Cyanotic operated	4S	39	4.46	2.77	-1.69	25	-6.79 <sup>b</sup>
Cyanotic nonoperated	4S	46	4.00	4.04	.04	16	.11

<sup>a</sup> Negative change signifies improvement<sup>b</sup> Mean changes from first to last test are significant using a single test level of 0.05

dren who had surgery became less anxious and pampered and protected their children less when compared with the mothers of children who did not have surgery (Table 6). The changes were statistically significant. It must also be noted that the mothers of acyanotic children who did not have surgery also became less protective during the period from the first to the last test. The significant change in this group appears to be related to the decline in symptoms in this group with age and with a possibly greater assurance by the mother of the acyanotic child who does not look as sick as the blue child.

#### *Comparison with earlier studies*

There are a limited number of longitudinal studies of development in children with congenital heart disease comparing preoperative and postoperative status. Although Lindtman (4) found relatively normal intelligence in his group of children with congenital heart disease 72% had an increase in IQ following operation while 28% showed a decrease. An increase of over 10 IQ points occurred in 23 postoperatively while an equivalent decrease was noted in only four. Brett & Kohler (2) on the other hand found a higher incidence of lower IQ in children with cyanotic congenital

heart disease and this group showed a post operative increase in intelligence quotient. Malaspina (8) recorded a slight increase in the intelligence quotient in a small group of children with congenital heart disease tested 6 to 24 months after operation.

In a qualitative evaluation of behavioral changes Landtman found less preoperative deviation from the normal and less postoperative change. These differences may be related to population variation or differences in testing procedures. Still, he described the overall behavior of 56 of 84 children as improved after operation.

Of a small group of 25 over protective mothers (4) 11 became less over protective after successful operation. This change is not as marked as in our study but does not differentiate between parental attitude of parents with cyanotic and acyanotic children.

In a recent study Drash & Money (3) were concerned with the decrease in postoperative IQ in 2 of 4 children but this concern is not borne out in studies of larger numbers. However the possibility is not eliminated that transient IQ deficit may be present in the early postoperative period which is no longer evident six months after operation when re evaluation was done in our study.

## BLADDER STONE DISEASE OF CHILDHOOD

### I. An epidemiological study

A. L. AUROBA, O. F. TANHA and D. N. GUPTA

From the Department of Pathology and Surgery, Maulana A. Ali Medical College and Associated Institutions and O. B. Panu Hospital, New Delhi, India

Calculus disease of the urinary tract has probably been known since antiquity. The most ancient evidence of this comes from Egyptian mummies (20). Ruffer described three vesical calculi found by Flinders Petrie in a Predynastic skeleton (20). In 1901 Elliot Smith discovered a calculus lying among the pelvic bones of the skeleton of a boy aged 15 years whose grave was exhumed at El Amarah in upper Egypt (11). Urinary calculi (stones) seem to have been fairly common in India (4, 10, 12). It was considered a dangerous ailment comparable to the God of death. The condition has been also described by Hippocrates (15), Celsus (24), Galen (11, 24), Albucasis (15, 24) and Morgagni (1). Once a common entity in Western Countries (13, 14, 21) it has practically disappeared from those countries presumably due to the rise in living standards. It continues to be a pediatric problem in the developing countries of South East Asia (2, 3, 6, 16, 17, 23) where poverty and poor hygiene still prevail.

The relationship of bladder stone disease of childhood to the more universally distributed renal calculus has not been clearly defined. The etiology and pathogenesis of this condition also

remain obscure. It was with this background that the present studies were undertaken.

### MATERIAL AND METHODS

Records of all proved cases of urinary calculi admitted to Safdarjung and Irwin Hospitals, New Delhi during the years 1963 and 1964 were studied. A case was considered proved if he/she had spontaneously passed the stone(s) in the hospital or the stone(s) was/were removed surgically or/and radiologically examination including intravenous pyelography clearly showed the presence of stone(s). All such cases were analysed as to the location of stone and age and sex of the patient.

The incidence of urolithiasis in relation to total surgical admissions and total attendance to the out-patient departments of the hospitals was recorded.

It may be pointed out that these two hospitals chiefly serve the poor and lower middle classes of the society of Delhi and its neighbouring areas.

Hospital records of St. S. Nursing Home, New Delhi, a large private Nursing Home catering to the needs of higher middle class and rich persons were similarly analysed for the years under study.

Forty-four cases of bladder stone in children were followed (by questionnaire) for a period of 1 to 10 years for any recurrence.

Heights and weights of children in the age group 5 to 14 years admitted to two schools in Delhi were recorded and compared with the heights and weights of bladder stone cases to assess deviation if any in the growth patterns to these three groups. Children of one school belonged to poor families and those of other school came from rich families.

Meteorological data was obtained from the Director Meteorological Department of India and seasonal incidence of disease was recorded. In making this analysis the time of occurrence of the disease was

Assistant Professor of Pathology  
Associate Professor of Surgery  
Professor of Pathology



## REFERENCES

- 1 Anastasi A *Standardized ability testing in P II* Musson (ed) *Handbook of Research Methods in Child Development* John Wiley & Sons New York 1960 p 456
- 2 Brett J & Kohler C Incidences neuropsychiatiques des cardiopathies congénitales chez l'enfant *Pediatr* 11 59 1956
- 3 Drish P W & Money J Statural and intellectual growth in congenital heart disease in growth hormone deficiency and in sibling controls in D B Cheek (ed) *Human Growth Body Composition Cell Growth Energy and Intelligence* Lea and Febiger Philadelphia 1968 p 606
- 4 Lindman II Vlatane E Pentti R & Anker M Psychosomatic behavior of children with congenital heart disease Pre and postoperative studies of eighty four cases *Ann Paediat Fenn* 61 1960
- 5 Linde L M Rasof B Dunn O J & Rabb E Attitudinal factors in congenital heart disease *Pediatrics* 38 92 1966
- 6 Linde L M Rasof B & Dunn O J Mental development in congenital heart disease *J Pediatr* 71 199 1967
- 7 Linde L M Dunn O J Schureson R & Rasof B Growth in children with congenital heart disease *J Pediatr* 70 413 1967
- 8 Malaspina M & Bono G Considerazioni sullo sviluppo mentale dei cardiopatici congeniti *Minerva Cardioangiol* 3 207 1955
- 9 Rasof B Linde L M & Dunn O J Intellectual development in children with congenital heart disease *Child Develop* 38 1043 1967

Submitted June 10 1969

Accepted Sept 21 1969

(L. M. L.) Dept of Pediatrics  
School of Medicine  
University of California  
Los Angeles  
California 900 24  
U.S.A

**Key words.** Congenital heart disease longitudinal studies intellectual development behavioral development

## BLADDER STONE DISEASE OF CHILDHOOD

### 1 An epidemiological study

A L. AURORA O P. TANEJA and D N. GUPTA

*From the Department of Pathology and Surgery Maulana Azad Medical College  
and Associated Iqbal and G B Pant Hospitals New Delhi India*

Calculus disease of the urinary tract has probably been known since antiquity The most ancient evidence of this comes from Egyptian mummies (20) Ruffier described three vesical calculi found by Flinders Petrie in a Predynastic skeleton (20) In 1901 Elliot Smith discovered a calculus lying among the pelvic bones of the skeleton of a boy aged 35 years whose grave was exhumed at El Amarah in upper Egypt (11) Urinary calculi (sauran) seem to have been fairly common in India (4 10 12) It was considered a dangerous ailment comparable to the God of death The condition has been also described by Hippocrates (15) Celsus (24) Galen (11 24) Avicenna (15 24) and Morgagni (1) Once a common entity in Western Countries (13 14 21) it has practically disappeared from those countries presumably due to the rise in living standards It continues to be a pediatric problem in the developing countries of South East Asia (2 3 5 16 17 23) where poverty and poor hygiene still prevail

The relationship of bladder stone disease of childhood to the more universally distributed renal calculus has not been clearly defined The etiology and pathogenesis of this condition also

remain obscure It was with this background that the present studies were undertaken

### MATERIAL AND METHODS

Records of all proved cases of urinary calculi admitted to Safdarjung and Iqbal Hospitals New Delhi during the years 1963 and 1964 were studied A case was considered proved if he/she had spontaneously passed the stone(s) in the hospital or the stone(s) was/were removed surgically or/and radiologically examination including intravenous pyelography clearly showed the presence of stone(s) All such cases were analyzed as to the location of stone and age and sex of the patient

The incidence of urolithiasis in relation to total surgical admissions and total attendance to the out patient departments of the hospitals was recorded

It may be pointed out that these two hospitals chiefly serve the poor and lower middle classes of the society of Delhi and its neighbouring areas

Hospital records of Sarda Nursing Home New Delhi a large private Nursing Home catering to the needs of higher middle class and rich persons were similarly analyzed for the years under study

Forty four cases of bladder stone in children were followed (by questionnaire) for a period of 1 to 9 years for any recurrence

Heights and weights of children in the age group 5 to 14 years admitted to two schools in Delhi were recorded and compared with the heights and weights of bladder stone cases to assess deviation if any in the growth patterns in these three groups Children of one school belonged to poor families and those of other school came from rich families

Meteorological data was obtained from the Director Meteorological Department of India and seasonal incidence of disease was recorded In making this analysis the time of occurrence of the disease was

Assistant Professor of Pathology  
Associate Professor of Surgery  
Professor of Pathology

Table 1 Prevalence of urolithiasis in the two sexes

Population studied (1963-64)	Kidney stone (All ages)		Kidney stone Children 0-14 years		Bladder stone (All ages)		Bladder stone Children 0-14 years	
	M	F	M	F	M	F	M	F
Poor population TA = 182040 M F = 12 1 SA = 44273 M F = 17 1	6	1	6	1	28	6	33	6
Well to do population TA = 1538 M F = 24 1 SA = 985 M F = 25 1	2	7	Male 0 Female 1 case		Male 3 cases Female 0		Male 0 Female 0	

TA = Total admissions  
SA = Surgical admissions  
M = Male  
F = Female

calculated from the date of commencement of symptoms clearly attributable to urolithiasis rather than the date of presenting at the hospital.

Sixty one cases of bladder stone disease in children were analysed as to the urban and rural distribution. Any relevant family or past history was recorded in these cases.

The data on hardness of water supplied to the population of Delhi and New Delhi was obtained from the Chief Water Analyst Delhi.

## RESULTS

A total of 1 952 774 patients attended the outpatient Departments of Irwin and Safdarjung Hospitals. The total number of admissions were 182 040 and of these 44 273 were admitted to the surgical wards. There were 396 cases of bladder stone and 233 cases of renal lithiasis.

In Sen's Nursing Home 1984 patients attended as outpatients. The total number of admissions was 1538 and of these 985 were admitted to the surgical wards. There were 3 cases of bladder stone and 18 cases of renal lithiasis.

The prevalence of urolithiasis in the two groups of population in the two sexes is given in Table 1. It is obvious that both renal and bladder calculi tend to be more frequent in males of the poor population than in the well

to do. Further the bladder stone cases are four to five times more frequent than kidney stones in the poor population. Bladder stones are rare in the well to do.

The incidence of calculi in various age groups is given in Fig. 1. It will be seen that in the poor population there are two peaks for bladder stone cases: one in the age group 0-10 years and the other after 45 years. The kidney stone cases show a gradual rise up to the age 20-24 years after which there is a gradual fall to be followed by another rise after the age of 45 years. The rise in both kidney and bladder stone cases after 45 years could possibly be due to obstructive uropathies. In the well to do there was no case of bladder stone in the 0-14 years age group and there were only 3 cases in the later ages. The kidney stone cases showed a trend very similar to those in the poor population.

The incidence of urolithiasis expressed as a percentage of surgical admissions in both sexes of the two population groups is shown in Fig. 2. It will be observed that in the poor population bladder stone cases in children are six times more frequent than kidney stones whereas bladder stone cases for all ages are nearly 1 1/2 times more frequent than the kid

ney stones. This situation is reversed in the well-to-do population where kidney stones were six times that of the bladder stones. Further it should be stated that the incidence of urolithiasis in the well-to-do population is higher than in the poor population when the data are expressed as percentages of the total and surgical admissions as given below.

Population	Percentage of total admissions	Percentage of surgical admissions
Poor	0.43	1.58
Well-to-do	2.27	3.54

The above epidemiological data regarding the prevalence of the disease clearly shows that bladder stone cases are more frequent in the poor population especially in the male children whereas the kidney stone cases are often observed in the well-to-do. Urolithiasis in

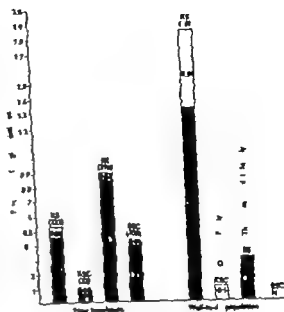


Fig. 2 Incidence of urolithiasis expressed as percentage of surgical admissions (SA). Male ■ Female □ Poor population total SA = 44.173 M F = 17.1 Well-to-do population total SA = 963 M F = 25.1 KS = Kidney stone KSC = kidney stone in children BS = bladder stone BSC = bladder stone in children Values in parentheses indicate the total no. of cases.

children is a rarity in the latter class of population.

#### Recurrence in bladder stone cases after surgical removal

Since 1935 44 cases of bladder stone disease who underwent surgery have been followed for a period varying from 1½ to 9½ years as shown in Table 2. It is significant to note that there was not even a single case with persistent or recurrence of symptoms of bladder stone disease. This shows that following surgical removal if recurrence does take place in

Table 2 Follow up of 44 cases of bladder stone in children

Duration of follow up (years)	No. of cases	Recurrence
1½ to 3½	20	NIL
3 to 7½	9	NIL
8 to 9½	15	NIL

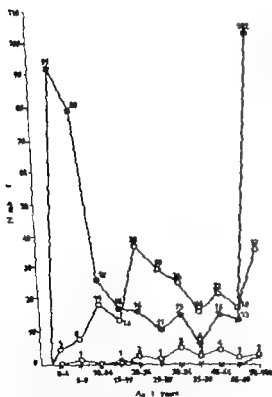


Fig. 3 Incidence of urolithiasis in various age groups. Bladder stone cases —●— poor population 396 cases — well-to-do population 3 cases. Kidney stone cases —○— poor population 233 cases — well-to-do population 18 cases.

Table 1 Prevalence of urolithiasis in the two sexes

Population studied (1963-64)	Kidney stone (All ages)	Kidney stone Children 0-14 years	Bladder stone (All ages)	Bladder stone Children 0-14 years
	M F	M F	M F	M F
Poor population TA = 182040 M F = 12 1 SA = 44273 M F = 17 1	61 1	63 1	28 6 1	33 6 1
Well to do population TA = 1538 M F = 24 1 SA = 985 M F = 25 1	27 1	Male 0 Female 1 case	Male 3 cases Female 0	Male 0 Female 0

TA = Total admissions  
SA = Surgical admissions  
M = Male  
F = Female

calculated from the date of commencement of symptoms clearly attributable to urolithiasis rather than the date of presenting at the hospital.

Sixty one cases of bladder stone disease in children were analysed as to the urban and rural distribution. Any relevant family or past history was recorded in these cases.

The data on hardness of water supplied to the population of Delhi and New Delhi was obtained from the Chief Water Analyst, Delhi.

## RESULTS

A total of 1 952 774 patients attended the outpatient Departments of Irwin and Safdarjung Hospitals. The total number of admissions were 182 040 and of these 44 273 were admitted to the surgical wards. There were 396 cases of bladder stone and 233 cases of renal lithiasis.

In Sen's Nursing Home, 1984 patients attended as outpatients. The total number of admissions was 1538 and of these 985 were admitted to the surgical wards. There were 3 cases of bladder stone and 18 cases of renal lithiasis.

The prevalence of urolithiasis in the two groups of population in the two sexes is given in Table 1. It is obvious that both renal and bladder calculi tend to be more frequent in males of the poor population than in the well

to do. Further the bladder stone cases are four to five times more frequent than kidney stones in the poor population. Bladder stones are rare in the well to do.

The incidence of calculi in various age groups is given in Fig. 1. It will be seen that in the poor population there are two peaks for bladder stone cases: one in the age group 0-10 years and the other after 45 years. The kidney stone cases show a gradual rise up to the age 20-24 years, after which there is a gradual fall to be followed by another rise after the age of 45 years. The rise in both kidney and bladder stone cases after 45 years could possibly be due to obstructive uropathies. In the well to do there was no case of bladder stone in the 0-14 years age group and there were only 3 cases in the later ages. The kidney stone cases showed a trend very similar to those in the poor population.

The incidence of urolithiasis expressed as a percentage of surgical admissions in both sexes of the two population groups is shown in Fig. 2. It will be observed that in the poor population bladder stone cases in children are six times more frequent than kidney stones, whereas bladder stone cases for all ages are nearly 1 1/2 times more frequent than the kid-

Table 4. Seasonal incidence of cases with bladder stone in children

Months	Present series 61 cases (1962-65)		Total cases studied 147 (1955-65)	
	No of cases	age of 61	No of cases	age of 147
January	3	19.7	13	23.2
February	2		9	
March	5		15	
April	4		9	
May	3	22.9	9	24.5
June	7		18	
July	3		9	
August	5	32.8	17	29.9
September	12		18	
October	4	26.6	9	20.4
November	9		14	
December	2		7	

*Family and past history*

The size of the family varied from three to six members. All 61 cases belonged to poor or lower middle class. Some 80.3% came from very poor families with an income of less than 150 rupees per month and 19.7% belonged to the lower middle class group with an income ranging from 151 to 400 rupees per month.

In none of these cases was a family history of bladder stone available. The father of one case and an uncle of another case gave histories of passage of stone per urethrum after attacks of renal colic. Past history of diarrhoea or infectious fever prior to the onset of symptoms of bladder stone disease was available in six patients. Two patients had episodes of diarrhoea 6 months before the onset of bladder stone disease. In two patients a history of measles 7 months and 10 months prior to the onset of symptoms of bladder stone was available. Two patients suffered from measles immediately before they had the symptoms attributable to bladder stone.

*Hardness of water*

The water supplied to the residents of Delhi and New Delhi has a hardness ranging from about 120 to 225 ppm (expressed as calcium

carbonate). This degree of hardness is well below the maximum total hardness of 600 ppm permitted for drinking water.

## DISCUSSION

*(a) Prevalence*

A high prevalence of bladder stone cases in contrast to renal calculi has been reported from Thailand (5, 6, 17, 23), South China (19) and India (2, 3, 22). In these studies the method of investigation has been either a population survey or an analysis of the hospital records. Pastmore (17), Sanong Unakul (23) and Halstead & Vallyasevi (6, 7) have reported high prevalence of bladder stone disease in north-east Thailand on the basis of population surveys. Sanong Unakul (23) in his extensive statistical survey of the problem of urolithiasis in Thailand reported an incidence of 0 to 10.29 per 10 000 for the entire country. In this series vesical calculi constituted 82.0% of all stones. Pastmore (17) quoting Dr Cholvin Chankom Hospital Director of Ubol Provincial Hospital states that in a period of 7 years whereas 1250 vesical calculi had been removed there were only one or two renal calculi each year. In his analysis of hospital records of Canton hospital in South China Thomson as quoted by Siddall (19) could find 2962 cases of vesical calculi as compared to only 5 cases of renal stone.

The prevalence of bladder stone disease in various parts of India has been highlighted by McCarron (16), Rao (18) and Andersen *et al* (2). The figures given by McCarron (16) for whole of India range from 0 to 43.8 per 10 000 population. Andersen *et al* (2) reporting on the bladder stone cases from Ahmednagar (1953-54) found an incidence of 0.85 per 10 000 population. The latter workers also studied 61 children with bladder stone from 1951-57. During this period 4452 children were admitted to the hospital giving an incidence of 1.3% of all pediatric admissions. Aurora & Ramalingaswami (unpublished data) collected figures for urolithiasis from various

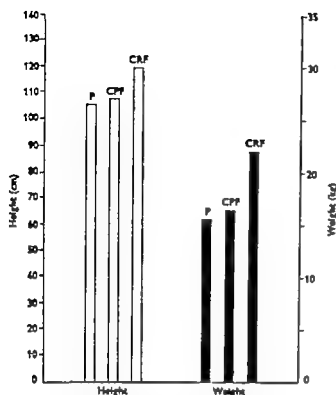


Fig 3 Growth pattern in cases of bladder stone disease and in control children. Age group 5-9 years. P = Bladder stone cases. CPF = children of poor families. CRF = children of rich families.

bladder stone cases of childhood it must be very rare.

#### Growth patterns in cases of bladder stone disease

This was assessed on the basis of heights and weights recorded in some of the patients. The data have been compared with school children

belonging to poor and rich families, who did not have any clinical evidence of bladder stone disease. The results are given in Fig 3. It will be noted that the growth patterns of the bladder stone cases and of the children from poor families are essentially similar. On the other hand, children from rich families had statistically better growth patterns than both the bladder stone cases and children from poor families. It is thus apparent that the growth pattern of bladder stone cases is in no way different from the generally poor population from which most of the cases come.

#### Seasonal prevalence of bladder stone cases

The meteorological data regarding the temperature, rainfall and relative humidity for the years 1962-1965 are given in a consolidated form in Table 3 and the incidence of bladder stone cases is recorded in Table 4.

It will be seen that there is no significant difference between the number of cases in summer and winter while many other cases had their first symptoms in the rainy season.

#### Urban and rural distribution of bladder stone cases

In 61 cases of bladder stone in childhood 52.5% of the cases belonged to the urban population while 47.5% came from rural population.

Table 3

Months	Temperature (°C)		Rainfall (mm)	Relative humidity (%)
	Maximum	Minimum		
January	19.2 to 22.6	4.5 to 8.6	0.0 to 33.2	75 to 80
February	23.8 to 26.5	9.1 to 11.5	1.5 to 12.6	67 to 77
March	29.3 to 32.1	14.0 to 16.2	1.0 to 8.0	45 to 60
April	33.3 to 37.0	19.3 to 22.5	0.0 to 13.9	38 to 50
May	38.4 to 40.8	23.5 to 28.3	0.0 to 16.0	35 to 41
June	38.4 to 40.8	27.1 to 28.7	3.0 to 117.6	38 to 57
July	33.0 to 37.6	24.5 to 27.6	45.8 to 538.2	68 to 83
August	32.4 to 34.4	24.9 to 26.0	84.6 to 446.3	72 to 87
September	32.3 to 34.2	22.9 to 23.7	181.5 to 277.7	66 to 79
October	32.7 to 33.6	16.9 to 20.1	0.0 to 0.2	57 to 63
November	27.7 to 29.8	10.6 to 14.9	0.0 to 2.0	59 to 68
December	21.6 to 23.2	6.1 to 7.8	0.1 to 27.4	55 to 82

low the age of 14 years and again after 50 years. In the old age groups the stones are nearly always secondary to obstructive uropathies.

The overall prevalence of urinary stones in males is high. The occurrence of bladder stone cases as compared with kidney stone cases in males is also distinctly more frequent. In the present series in the poor population the male to female ratio was high especially in case of bladder calculi.

The higher incidence of bladder stone cases in males has been explained on the basis of the anatomical difficulty for the escape of stone presented by the male bladder neck and by the long tortuous urethra. In order to test the validity of this hypothesis zinc spheres 1.8 cm or 1.2 cm in diameter were introduced in the bladders of male rabbits. Of the four spheres introduced three were expelled and one was retained. The two smaller spheres of 1.2 cm were expelled in 3 to 4 days and of the two larger spheres one was expelled in 42 days. It may be mentioned that the larger sphere which was retained was heavier than the similar sized sphere which was expelled. This becomes of considerable significance when it is realized that human bladder has much greater musculature and the male urethra in humans is certainly wider than the rabbit urethra. Furthermore the zinc spheres used in the animal experiments were much larger than many of the stones recovered from the bladders of children. Thus a long narrow urethra in the male *per se* does not explain the higher frequency of bladder stone in this sex. Again it is not only in the bladder but also in the case of kidney stones that the prevalence in males is higher than in females. For the retention of stones in the urinary tract it is probable that the stones in early stages stick to the urothelium so as not to be expelled by the stream of urine or contractions of the bladder. The explanation for the higher prevalence in males may be the possible role of sex hormones deficiency of some calculus inhibitor in the urine and role if any of the prostate gland.

## SUMMARY

An epidemiological study of cases of urolithiasis in poor population and affluent society was undertaken. The study revealed a great preponderance of bladder stone cases in childhood in the poor population as against its marked paucity in the well-to-do. The condition occurred predominantly in the male children. The follow up studies show that bladder stones in children do not recur after surgical removal. This study fails to show any seasonal relationship in the prevalence of this malady. In the present series there was no family history of bladder stone and the cases were nearly equally distributed among the urban and rural population. The findings of the present study are discussed in the light of the pertinent literature.

## ACKNOWLEDGEMENTS

We are grateful to the Medical Superintendents of Sairajyog Hospital and Sena Nursing Home for permission to use the data of the patients admitted.

## REFERENCES

- 1 Alexander B. *The seats and causes of diseases investigated by anatomy*. Hafner Publishing Co. New York 1960 Vol II Letter XLII.
- 2 Andersen D A, Srinivachari S & Khandepale M K. Investigations into relationship between bladder stones and malnutrition. *Indian J Med Sci* 17 617 1963.
- 3 Aurora A L, Ramalingaswami V & Gaziode P D. Bladder stone disease in children in Delhi area. *J Urol* 91 347 1964.
- 4 Castiglione A. *A history of medicine*. Translated from the Italian and edited by E B Krumbhaar. Routledge & Kegan Paul London 1947 p 97.
- 5 Halstead S B. Bladder stone in Thailand. A review of the problem. *Amer J Trop Med* 10 918 1961.
- 6 Halstead S B & Vajpayee A. *Clinical and epidemiological aspects of renal calculi disease in Thailand and elsewhere*. In compilation of papers presented at a workshop on clinical and experimental urolithiasis held at the Naval Medical Research Institute National Naval Medical Center Bethesda Maryland on 8 and 9 June 1965.
- 7 — Studies of bladder stone disease in Thailand I. Introduction and description of area studied. *Amer J Clin Nutr* 20 1317 1967.
- 8 — Studies of bladder stone disease in Thailand



hospitals in India. High incidence of urolithiasis was reported from Moradabad district, where a visit showed the abundance of renal lithiasis and only few cases of bladder stone in children. This is in contrast to high prevalence of bladder stone cases in some areas of Thailand.

The epidemiological data collected from three large hospitals in the present series shows that bladder stone cases constitute nearly half of all cases of urolithiasis and of these nearly 50% occur in children (0-14 years). The data from Saldarjung and Irwin Hospitals which cater for the poor population show 47.3% of bladder stone cases in children compared with 12.47% of kidney stone cases. The incidence of bladder stone and kidney stone cases expressed as a percentage of the pediatric surgical admissions was 1.62% and 0.26% respectively. On the other hand the data from Sens Nursing Home hospital which serves the affluent society revealed that whereas no case of bladder stone occurred in children (0-14 years) one case of kidney stone was observed in this age group. Thus in children of privileged class urolithiasis is rare. This trend is similar to the observations made in the developed nations of the world, once a common malady in the children of Western countries it is now a rarity with the rise in living standards. The paucity of bladder stones in children in areas where renal lithiasis abounds and vice versa suggests that the factors operating in renal lithiasis are not directly concerned in the genesis of primary bladder stone disease of childhood.

#### (b) Rural and urban prevalence

Passmore (17) and later Halstead (5) and Halstead & Valyasev (8) have indicated the rarity of bladder stone cases in urban areas and their high prevalence among the rural population of Ubol Province (Thailand).

In the present series though no comparable population surveys were conducted the data strongly suggest that the prevalence of bladder stone cases in urban and that in rural popula-

tion are similar. In this study 52.5% of 6 cases belonged to the urban population while 47.5% came from rural areas. It is probable that the children of the poor urban population who chiefly suffer from this disease live under conditions similar to those of rural population.

#### (c) Climatic factors

Dehydration consequent on excessive sweating in summer leading to the concentration of urine and thus disturbing the crystalloid colloid ratio has been implicated in the genesis of stone (9). The study of 61 cases of present series and 147 cases over the last 10 years fails to show greater incidence of the disease in summer. In the evaluation of this data the time of occurrence of the disease was calculated from the date of commencement of symptoms clearly attributable to urolithiasis rather than the date of presenting at the hospital. It would have been observed that there is slightly greater incidence of disease during rainy season due to rather more cases in September in the present series. It might be argued that symptoms appear when the stone has attained a certain size and the duration of symptoms may not necessarily indicate the time of intubation of the stone. Thus dehydration might be implicated indirectly as a factor in the genesis of stone. If this be so then there is no reason why recurrence should not be frequent in the patients who have once formed stone.

#### (d) Family history

Halstead & Valyasev (6) in their studies from Thailand state that the persons who shared the same food and household environment as the stone cases were twice as likely to develop stones as the average. In the present series in none of the cases was a family history of bladder stone available.

#### (e) Age and sex

The data in the present series are comparable to the series reported by other workers (2, 22). Maximum numbers of cases are observed be-

## CONGENITAL HYPOPLASTIC THROMBOCYTOPENIA AND CEREBRAL MALFORMATIONS IN TWO BROTHERS

HANS M. HØYERAAAL, JON LAMVIK and PETER JOHAN MØR

From the Department of Paediatrics and the Oslo Institute Department of Pathology, University of Bergen, Norway

At least two syndromes comprising disturbed blood cell production and congenital abnormalities have been reported.

In 1927 Fanconi (7) described three brothers 5-7 years of age who developed chronic pancytopenia which proved fatal within a year of onset. Patients with Fanconi syndrome often have multiple congenital anomalies: abnormal pigmentation of the skin, stunted growth, strabismus, exaggerated reflexes and microcephaly. Aplasia of the radius is only reported in a few cases (3, 11).

Isolated congenital hypoplastic thrombocytopoenia associated with one or more anomalies has been reported in at least 35 cases (4, 5, 8, 12, 15, 18) since Greenwald & Sherman in 1929 reported the first case (9). Cases with known infectious viral infections have been excluded. Aplasia of the radius seems to occur in about 1/4 of the cases. In fact an attempt has been made to separate a special syndrome consisting of aplasia of the radius, megakaryocytic thrombocytopoenia and leukemoid reaction from the other cases of congenital hypoplastic thrombocytopoenia (1, 6, 13). The most frequent other abnormalities are other skeletal defects, cardiac and urogenital anomalies.

Only one case of persistent congenital hypoplastic thrombocytopoenia associated with microcephaly has been found in the literature (5).

The purpose of the present report is to describe the clinical, hematological and pathological findings in two brothers with congenital hypoplastic thrombocytopoenia, microcephaly, with cerebellar atrophy and a probable hemolytic anemia.

### CASE REPORTS

**Case 1** S. M., a male infant born on the 4th September 1958 following an uneventful pregnancy of 41 weeks. The economic find was foetal molding and good. Birth weight was 2400 g and length 45 cm. He was unresponsive but cried after 12 min following resuscitation. At the age of three months he was admitted to the Children's Hospital, Bergen because of failure to thrive. He was microcephalic (largest head circumference of 35.5 cm, 2 cm below the 2% percentile for age) and had lateral strabismus of the eyes. Motor activity was decreased whereas muscular tone was increased. No reflex abnormalities were elicited. Routine blood investigations and EEG revealed no abnormalities. Roentgenograms of the skull showed prominence of the sphenoid suture. At that time no definite diagnosis was made.

At the age of 4 months he had a left-sided torticollis corrected with plaster of Paris. He burred readily from the age of 9 months. Psychomotor development was retarded.

On his second admission at the age of 21 months he gave the impression of an intellectual retardation. His length, 75 cm, was 3 cm below the 2% percentile for age; his weight, 7.7 kg, was 0.7 kg below the 2% percentile for length. He was 47 cm below the 2% percentile for age. He was spastic particularly in the lower extremities. Multiple ecchymoses and petechiae were seen. The platelet count using Haremon's method (13) varied between 13,000 and 41,000 per mm<sup>3</sup>. The bleeding time (finger pricks) was markedly prolonged (the

- III Epidemiological studies in Ubol Province  
*Amer J Clin Nutr* 20 1329 1967
- 9 Harlin H C A logical approach to the problem of urinary calculi prophylaxis *New York State J Med* 55 213 1955
  - 10 Joly J *Indian Medicine* C G Kashukar (Tilak) Poona (India) 1951 pp 123-125
  - 11 Joly J S The etiology of stone *J Urol* 32 541 1934
  - 12 Kutumbiah P *Ancient Indian Medicine* Orient Longmans Calcutta 1962 p 167-168
  - 13 Lett H On urinary calculus with special reference to stone in the bladder *Brit J Urol* 8 205 1936
  - 14 Lowale O S & Kirwin T J *Clinical Urology* Williams & Wilkins Baltimore 1940 Vol II pp 1025-6
  - 15 Mettler C C *History of Medicine* Blakiston Philadelphia 1947 p 696
  - 16 McCarrison R A lecture on the causation of stone in India *Brit Med J* 1 1009 1931
  - 17 Prusmore R Observations on the epidemiology of stone in the bladder in Thailand *Lancet* I 638 1953
  - 18 Rao M V R *Report on the studies on vesical calculus (stone in the urinary bladder) in Mehsana District (1953-55)* Director Govern-  
ment Printing Publications and Stationery Bom-  
bay State Bombay 1958
  - 19 Siddall A C Primary vesical calculus *J Urol* 37 268 1937
  - 20 Sigerist H E *A history of Medicine* (Vol I Primitive and Archaic Medicine) Oxford Uni-  
versity Press New York 1955 p 63
  - 21 Thomas J M R Vesical calculus in Norfolk  
*Brit J Urol* 21 20 1949
  - 22 Tiwari C B Bladder stones in children *Indian  
Practitioner* 11 1055 1958
  - 23 Unakul S Urinary Stones in Thailand A sta-  
tistical survey *Sriraj Hospital Ga zette* 13 199  
1961
  - 24 Winsbury White H F *Stone in the urinary  
tract* Butterworth London 1954 2nd edition
- Submitted July 20 1969  
Accepted Sept 29 1969
- (A L A) Dept of Pathology  
Maulana Azad Medical College  
New Delhi-1  
India
- Key words Bladder stone disease children epidemo-  
logical study

## CONGENITAL HYPOPLASTIC THROMBOCYTOPENIA AND CEREBRAL MALFORMATIONS IN TWO BROTHERS

HANS M. HOYERAAAL, ION LAMVIK and PETTR JOHAN MOE

From the Department of Paediatrics and the Gade Institute Department of Pathology, University of Bergen, Norway

At least two syndromes comprising disturbed blood cell production and congenital abnormalities have been reported.

In 1927 Fanconi (7) described three brothers 5-7 years of age who developed chronic pancytopenia which proved fatal within a year of onset. Patients with Fanconi syndrome often have multiple congenital anomalies: abnormal pigmentation of the skin, stunted growth, strabismus, exaggerated reflexes and microcephaly. Aplasia of the radius is only reported in a few cases (3, 11).

Isolated congenital hypoplastic thrombocytopoenia associated with one or more anomalies has been reported in at least 35 cases (4, 5, 8, 12, 14, 18) since Greenwald & Sherman in 1929 reported the first case (9). Cases with known intrauterine viral infections have been excluded. Aplasia of the radius seems to occur in about 1/3 of the cases. In fact, an attempt has been made to separate a special syndrome consisting of aplasia of the radius, megakaryocytic thrombocytopoenia and leukemoid reaction from the other cases of congenital hypoplastic thrombocytopoenia (1, 6, 15). The most frequent other abnormalities are other skeletal defects, cardiac and urogenital anomalies.

Only one case of persistent congenital hypoplastic thrombocytopoenia associated with microcephaly has been found in the literature (5).

The purpose of the present report is to describe the clinical, hematological and pathological findings in two brothers with congenital hypoplastic thrombocytopoenia, microcephaly with cerebellar atrophy and a probable hemolytic anemia.

### CASE REPORTS

**Case 1:** A male infant born on the 4th September 1958 following an uneventful pregnancy of 41 weeks. The amniotic fluid was foul smelling and green. Birth weight was 2400 g and length 45 cm. He was apneustic but cried after 12 min following resuscitation. At the age of three months he was admitted to the Children's Hospital, Bergen because of failure to thrive. He was microcephalic (largest head circumference of 33.3 cm, 2 cm below the 2% percentile for age) and had lateral slanting of the eyes. Visual activity was decreased whereas muscular tone was increased. No reflex abnormalities were elicited. Routine blood investigations and ECG revealed no abnormalities. Roentgenograms of the skull showed prominence of the sigmoid suture. At that time no definite diagnosis was made.

At the age of 4 months he had a left motor proequinovarus corrected with plaster of Paris. He bawled readily from the age of 9 months. Psychomotor development was retarded.

On his second admission at the age of 21 months he gave the impression of mental retardation. His length 75 cm was 3 cm below the 2% percentile for age, his weight 7.7 kg was 0.7 kg below the 2% percentile for length whereas his head circumference 41 cm was 4.7 cm below the 2% percentile for age. He was apneustic particularly; the lower extremities had multiple ecchymoses and petechiae were seen. The platelet count using Kristensen's method (13) varied between 13 000 and 41 000 per mm<sup>3</sup>. The bleeding time (finger puncture) was markedly prolonged, the



Fig. 1. Photograph of case 2 at the age of 20 months.

coagulation and prothrombin times were normal. There was no anemia or leucopenia but relative granulocytosis. The erythrocyte sedimentation rate ranged from 41 to 72 mm per hour. Occult blood was found on repeated stool examinations. Bone marrow smears were reported to show abundant megakaryocytes. He died almost 23 months old, 36 hours after a head injury. Prior to death peripheral blood smears revealed 23 nucleated red blood cells per 100 white blood cells.

*Autopsy* revealed a subdural hematoma. The brain was small, 610 g, with a very small cerebellum weighing only 1.5 per cent of the whole brain. The lateral cerebral ventricles were dilated, particularly in the posterior part. The vermis as well as the cerebellar hemispheres were hypoplastic with a thin cortex. Microscopically there was atrophy of the granular layer while the Purkinje cells were present in normal number, many appeared to be displaced into the molecular layer. The dentate nucleus showed no signs

of atrophy. Bone marrow sections from femur and tibia showed a differentiated cell picture but very few megakaryocytes were scanty.

*Case 2 N O M*, a younger brother of case 1, was born on 26th April 1965. In 1960 his mother had an abortion in the 4th month. Her uterus contained several myomas. In the present gestation of 40 weeks she was given regular norethisterone (Primobol N) injections from the 2nd to the 7th month but no thiazide therapy. The amniotic fluid was yellow. Birth weight was 2200 g and length 43 cm. The delivery and neonatal period were uneventful. Psychomotor development was retarded. He was admitted to the Children's Hospital as his mother felt that he might have the same disease as his brother E. He had febrile bursts from the age of 5 months and was very small.

On admission at the age of 19 months his length 74.5 cm corresponded to the 2 / percentile for age and his weight 7.6 kg was 1 kg below the 2 / percentile for length. He was pale, microcephalic, spastic and had a similar peculiar appearance (Fig. 1) to his brother. The largest head circumference was 42 cm, 3 cm below the 2 / percentile for age. A cerebellar type of ataxia was also noted. He had several purpuric lesions of the skin and mucous membranes but no lymphadenopathy or hepatosplenomegaly.

#### Laboratory investigations

His hemoglobin concentration was 8 g per 100 ml. The platelet count using Kristensen's method (11) ranged from 42 000 to 16 000 per mm<sup>3</sup>. The bleeding time (finger puncture) was markedly prolonged whereas the coagulation and prothrombin times and Heston test were normal. Few megakaryocytes were seen on repeated bone marrow smears. These were both large and small, mostly immature forms.

Röntgenograms of the skeleton showed retarded bone development. Air-encephalograms showed marked dilatation of the lateral ventricles, cortical atrophy and increased width of the septum pellucidum, most probably caused by a noncommunicating cyst.

Amino acid chromatography revealed increased urinary histidine (and alanine) excretion and low serum valine, leucine and isoleucine concentration (Dr S. Halvorsen, Pediatric Research Institute, Rikshospitalet, University of Oslo and Dr L. Gjovang, Dilemark Hospital, Asker). Chromosome studies showed a normal male karyotype (46 xy). Thrombocyte antibodies (complete and complement fixation) were not found (Dr K. Myrnes-Nielsen, Immunohematological Department, Kommunehospitalet, Aarhus). Platelet survival studies with transfused Cr-labelled platelets showed a normal life span of 8.9 days (Dr A. Fosb. Abrahamson, Oslo City Hospital and Dr K. Halvorsen, Immunohematological Department, University of Bergen).

Periodically there were signs indicating a hemolytic process with a sudden fall in hemoglobin concentration and an increase in the number of reticulocytes.

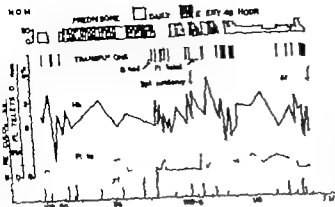


Fig 2 Case 2 Course from the age of 19 months to 3 years showing therapy and main hematological data

Nucleated red cells were also found in peripheral blood smears. He was slightly anemic several times serum bilirubin was 1.3 mg per 100 ml. Osmotic fragility curves only once revealed slightly increased fragility. Spherocytes were not seen. Direct Coombs test remained negative. Serum values of vitamin B<sub>12</sub> and folic acid were normal. Serum haptoglobins were 70-80 mg per 100 ml. Hemoglobin moved in the A and A fraction during disc electrophoresis.

Enzymatic studies of the red blood cells at 25°C and pH 7.5 showed normal activity for the following enzymes: hexokinase, phosphotransferase, phosphofructokinase, pyruvate kinase, lactate dehydrogenase, glucose 6-phosphate dehydrogenase and glutathione reductase. Adenosine nucleotides were also normal. These studies were carried out by Dr M. Hjaltn, The Acta Hospital, Uppsala.

Erythrocyte survival studies with <sup>51</sup>Cr labelled cells showed a half life time of 13 days of the patient's erythrocytes in a normal recipient (Dr L. Halvorsen, Immunohaematological Department, University of Bergen). Scanning of liver, spleen and bone marrow following autotransfusion of <sup>51</sup>Cr labelled red blood cells suggested the spleen as the main destruction site (Dr A. E. Eggen, Clinical Physiological Department, University of Bergen).

#### The age and course

The patient received prednisone therapy most of the time for years without definite effect on the thrombocytopenia. Nearly half the observation time he was on prednisone every other day (Fig 2). Repeated courses of supplementary therapy with anabolic steroids had no demonstrable clinical or hematological effect. Prednisone was discontinued mainly because of possible beneficial effect on the clinical condition, bleeding tendency and the hemolytic process. Because of hematocrit and anemia he periodically required blood transfusion (Fig 2). Five platelet transfusions gave only short lasting effect on the platelet counts.

During his last 1 year of life he suffered from recurrent otitis, gastroenteritis and episodic stomatitis (increasing brown skin pigmentation was seen).

Four months after having had chickenpox he had

herpes zoster. Gamma globulin quantitation by radial diffusion technique in agar revealed a hypo IgG (450-550 mg per 100 ml). IgM was slightly increased (Dr O. Tønder, Microbiological Department, University of Bergen). Test for antibody to toxoplasma in indirect hemagglutination gave pathological titer (160) but the Dye test was less than 4. Roentgenograms of the skull revealed no calcifications and no evidence of toxoplasmosis was found on examination of the eye. Splenectomy was performed in December 1967 in an attempt to control the hemolytic process and possibly influence the thrombocytopenia. The weight of the spleen was 55 g. Histologically the spleen showed hypertrophy. Two pigment deposits were seen. During the last months of life the patient had recurrent febrile periods. He received several courses of antibiotic therapy except terminally. He died at the age of 3 years.

Autopsy revealed a small brain 613 g against the normal weight of 1150 g. The macroscopical appearance was almost identical to that found in Case 1. The cerebellum was hypoplastic weighing only 1/3 of the normal weight with small symmetrical hemispheres and vermis. There was cerebral oedema with thickened meninges which showed macroscopical signs of subacute inflammation. The posterior parts of the lateral cerebral ventricles were dilated, most marked on the right side. A septum pellucidum cyst (Fig 3) was found with an anteroposterior diameter corresponding to the whole length of corpus callosum, which was quite thin while the anterior commissure and the fornix appeared normal.

No definite macroscopical abnormality was noticed in the cerebrum. The cerebral cortex was thin with an atrophic granular layer (Fig 4). The Purkinje cells were numerous, many appeared displaced into the molecular layer. Some showed club-like expansions or dendrites. The dentate nuclei and the inferior olives appeared normal.

The thymus was small and to a great extent replaced by fat. There was a lack of cortico-medullary differentiation. The lobes were composed of spindle formed cells with scattered Huxley's corpuscles.

There was hemorrhages into the skin and endocrines



*Fig 3* Case 2 Front section of the cerebrum showing the cystic cavity in the septum pellucidum and the dilated lateral ventricles

dium, left kidney and pelvis and the bone marrow was hypoplastic with very few megakaryocytes.

The post mortem examination in addition revealed giant cell pneumonia, centrolobular hepatic degeneration, moderate renal tubular degeneration and adiponecrosis around the pancreas.

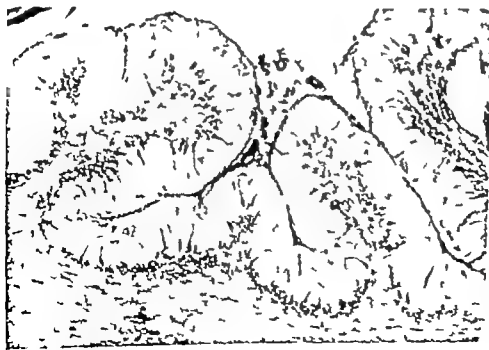
#### *Family studies*

The parents of the reported siblings, an older brother and a sister born in 1967, were all healthy. Clinical, hemitological and marker gene studies were performed in 26 members of the family. Five of them

gave a history of bruising readily but only one platelet counts (142 000–104 000 per 100 mm<sup>3</sup>) low normal range. One had congenital defect of palate. This of course could just as well have occurred in a random population.

The younger sister of the reported cases and 10 other members of the family had IgG below 800 per 100 ml (450–750 mg per 100 ml).

Owing to the positive hemagglutination test in 12 of all the 26 members of the family were tested antibodies to toxoplasma. The mother and three of the cousins had a positive hemagglutination and Dye



*Fig 4* Case 2 Microscopic section of the cerebellar hemisphere showing atrophy of the granular layer and prominent Purkinje cells. Htx+E  $\times 60$

The younger sister like case 2 had a positive hemagglutination test, but a negative Dye test. Two other members of the family had questionable positive tests.

No abnormality or inconsistency was found of the ABO Rh MN cell groups (Dr C Bruns Hansen Immunohaematological Department University of Bergen) Gc and Hp types (Dr Rønnekleiv Department of Forensic Medicine University of Oslo) Gm typing (Dr J B Natvig Rheumatological Research Department University of Oslo) and PGM typing (Dr E Moss Department of Forensic Medicine University of Oslo).

## DISCUSSION

—: reported siblings were very similar. They were both small for dates. They bruised readily from the age of a few months. had chronic thrombocytopenia, microcephaly, retarded growth, mental retardation, a peculiar appearance and spasticity. Autopsy revealed a small brain and a hypoplastic cerebellum in both. A cerebellar atrophy of the granular layer type (14) was evident suggesting destruction or lack of formation of the afferent neurons.

The normal survival of homologous platelets and lack of antibodies suggest thrombocytopenia due to insufficient production. The few megakaryocytes in the bone marrow smears from case 2 appear to be compatible with this assumption. Case 1 had abundant megakaryocytes in bone marrow smears when alive, scanty in bone marrow sections at autopsy. These findings in two siblings suggest that there is actually very little if any difference between cases of hypoplastic thrombocytopenia and the cases of amegakaryocytic thrombocytopenia. The same impression is gained from the literature (4, 10, 12, 18, 21).

Even the morphology of the megakaryocytes may be an unreliable criterion of lack of platelet production. Myllylä *et al.* (10) for instance demonstrated normal number of megakaryocytes without any clear morphological abnormality in 2 of their 15 cases of "hereditary thrombocytopenia" considered to be due to a defect in platelet production.

These two siblings seem to be the first reported cases of congenital hypoplastic thrombocytopenia associated with hypoplasia of the

cerebrum and cerebellum. One may speculate as to whether this represents a new syndrome or if it is just a variant of the previously reported cases of hypoplastic thrombocytopenia. Eisenstein published in 1966 (5) a prematurely born infant with congenital amegakaryocytic thrombocytopenic purpura. Psychomotor development appeared normal yet he was microcephalic, had a short stature and was unquestionably spastic. He had no ecchymoses or petechiae when last seen at the age of 22 months and his platelet count was at that time 80 000 per mm<sup>3</sup>.

No skeletal defects were found in the reported siblings and some of the clinical features (microcephaly, retarded growth and mental development, exaggerated deep tendon reflexes and pigmentation) are more in common with Fanconi anemia than with hypoplastic thrombocytopenia. Cerebral hypoplasia associated with severe hypoplasia of the cerebellum does not seem to have been reported in Fanconi syndrome either. No signs of erythropoietic hypoplasia were seen in the reported siblings.

At least in our second case the syndrome seems to include a hemolytic process. Definite decreased erythrocyte survival time when the patient's erythrocytes were injected into the circulation of a healthy person suggest an intracorpuscular defect. Zetterstrom & Strömberg (21) reported in 1958 two cases of "Sporadic Congenital Spherocytosis Associated with Congenital Hypoplastic Thrombocytopenia and Malformations". In our case 2 no spherocytosis or enzymatic defect were demonstrated. Increased susceptibility to infections in case 2 may be due to low serum levels of IgG (corticosteroid therapy and/or splenectomy). Although the thymus in case 2 was very small and atrophic this may presumably be secondary to a protracted wasting disease.

Fanconi anemia is considered to be due to an autosomal recessive gene with variable penetrance (11, 19, 20). A genetic basis for the occurrence of multiple cases of thrombocytopenia also seems unquestionable. Actually



## SODIUM POTASSIUM AND WATER CONTENT OF HUMAN FETAL AND MATERNAL PLASMA AND RED BLOOD CELLS

B BENGTTSSON G GENNER and E NILSSON

*From the Department of Pharmacology and the Department of Gynaecology and Obstetrics at the General Hospital of Malmö University of Lund Sweden*

The human fetal erythrocyte differs from its adult counterpart not only in that it contains predominantly hemoglobin F but also in other respects its mean life span is shorter presumably owing to factors inherent in the red blood cell (5) it is larger than the adult one (25) and its osmotic resistance, which is low during early fetal life increases towards partus (24) Important quantitative differences in lipid composition between the fetal and the adult erythrocyte have also been reported (6 13) Since almost all human erythrocyte lipids reside in the hemoglobin free ghosts (and therefore presumably in the membrane) functional differences between fetal and adult cells e.g. in electrolyte transport may be expected In this connexion intracellular concentrations of sodium and potassium are of obvious interest

McCance and Widdowson analysed erythrocytes from three human fetuses 18 to 20 weeks old and found a slightly lower potassium concentration in fetal red blood cells than in adult red blood cells (20) It was suggested that fetal cells were less able than adult cells to maintain a high potassium concentration gradient across the red cell membrane (30 31) However the potassium concentration in erythrocytes from newborns has been reported to be equal to or even greater than, that in adult red blood cells (1 23 32) Thus available data seem to suggest ontogenetic alterations in the intracellular cation concentrations in human fetal erythro-

cytes during the latter half of gestation but further information was considered necessary on this point

It has been reported that the potassium concentration in plasma from human fetuses at mid gestation and at term substantially exceeds the upper limit of the normal adult range (1 15 16 29 30) In a recent review it is stated the fetus seems to flourish with a concentration of potassium in its extracellular fluids well above that which is usually regarded as being the most efficient for the adult (31) However several reports have described a plasma potassium level in cord blood from newborns within or only slightly above the adult range (8 21 22 23) Furthermore in a recent study the plasma potassium concentration in umbilical artery of mid term human fetuses was found to lie within the normal adult range and only slightly above the maternal level (27) It was therefore considered of great interest to re-investigate the question of plasma potassium level in human fetuses and newborns with special reference to various factors in the sampling procedure which might explain discrepancies between earlier results The present report describes determinations of sodium and potassium in plasma and erythrocytes from human mid term fetuses and newborns and their corresponding mothers and from 1 day old infants

Table 1 Sodium and potassium in plasma and erythrocytes (RBC)  
mEq/kg mean  $\pm$  SEM

	Plasma		RBC		
	K	Na	K	Na	
I Mid-term fetus	4.81 $\pm$ 0.20	140.0 $\pm$ 0.7	99.9 $\pm$ 2.3	5.55 $\pm$ 0.1	n = 6
II Mother (fetus)	4.31 $\pm$ 0.09	135.1 $\pm$ 0.9	94.0 $\pm$ 1.6	7.26 $\pm$ 0.45	n = 6
III Newborn infant	4.59 $\pm$ 0.12	143.0 $\pm$ 1.3	97.0 $\pm$ 1.1	9.63 $\pm$ 0.58	n = 10
IV Mother (new-born infant)	3.66 $\pm$ 0.07	140.2 $\pm$ 1.1	91.5 $\pm$ 0.7	7.03 $\pm$ 0.28	n = 10
V 1-day-old infant	4.63 $\pm$ 0.04	140.3 $\pm$ 0.1	97.3 $\pm$ 0.5	10.26 $\pm$ 0.25	n = 10

RBC values corrected for 4.5 trapped plasma and 3.6 lost water during washing and centrifugation. Sodium and potassium values as expressed above (mEq/kg) may be converted to mEq/l wet weight by multiplying by 1.06 for red blood cells and by 1.07 for plasma.

Highly significant differences ( $p < 0.001$ )

Plasma K: Newborn infant > Mother (newborn infant)

RBC Na: Mid-term fetus < Newborn infant

Mid-term fetus < 1-day-old infant

Newborn infant > Mother (newborn infant)

## MATERIAL AND METHODS

### Collection of blood samples

Blood was collected from a series of 10 newborns at normal deliveries without clinical signs of intra-uterine asphyxia. The birth weights ranged 3000–4750 g and the Apgar score in the first 60 sec 9–10. The blood sample was separated from one umbilical artery immediately after delivery of the infant, concurrently with collection of blood from a maternal cutaneous vein. The blood samples were brought into heparinized glass tubes and the analysis was started at once.

A second series consisted of blood samples from 10 healthy newborns taken on the day after a normal delivery (see 5–29 hours birth weights 30.0–4440 g). Blood was obtained by catheterizing the umbilical vein up to the inferior caval vein after a laceration of the umbilical stump. Samples were only taken from vigorous infants without clinical signs of asphyxia.

A third group of samples were taken at laparotomy 14 hours performed by laparotomy. P.H. carcasses was given to the mothers with isoperitoneal sodium intravenous and theolone. The pre-natal age of the fetuses ranged 16–22 weeks and the fetal crown-rump length 17–53 cm. No fetal malformation or disturbance of the amniotic fluid was seen in this group. As soon as possible after incision of the uterus, loop of the umbilical cord was clamped. With the fetus left in situ one umbilical artery was catheterized and blood samples separated in portions of 1.2 ml. The exact time from incision to collection of each blood specimen was noted. Only cases where the time to first sample did not exceed 3 min were included in several instances only blood for a first sample could be obtained. Because of difficulty of getting enough fetal blood determinations of water content in plasma and erythrocytes in this series were performed on samples of mixed blood

from both umbilical vein and artery. A blood specimen was separated from a maternal vein of the mother concomitantly with the incision of the uterus.

### Determination of sodium and potassium

Plasma and erythrocytes were immediately separated by centrifugation in room temperature ( $2000 \pm 3$  mm). Samples showing haemolysis were discarded. Plasma was diluted 51 times (100  $\mu$ l + 5 ml distilled water) for analysis for potassium and a further 11 times (1 ml of the diluted plasma + 10 ml distilled water) for analysis for sodium.

After the initial centrifugation plasma and buffy coat were removed and the erythrocyte washed twice in an equal volume of choline Ringer solution composition (mM): choline-Cl 146, KCl 5, CaCl<sub>2</sub> 1, NaCl 5, orthophosphoric acid 2.5. This buffer to pH 4. This solution was used because Na-efflux from erythrocytes is maximum at an external Na concentration of approximately 5 mM (12). After each resuspension in the choline Ringer the erythrocytes were collected by centrifugation in room temperature ( $2000 \pm 3$  mm). The amount of plasma trapped among the erythrocytes at this centrifugal force is 4.5% as estimated from sucrose-C determinations (\*). A 'bar of 4.5' was used when correcting the determinations of intracellular sodium and potassium concentration for extracellular trapped fluid among the packed erythrocytes.

Extension of centrifugation time beyond 3 min did not change the amount of trapped extracellular fluid as judged from determinations of intracellular water content. Washing the erythrocytes twice was found to be sufficient, since further washing did not alter the value found for intracellular sodium concentration. The weight of 100  $\mu$ l washed erythrocytes was  $108.1 \pm 3.3$  mg,  $n = 20$  (mean  $\pm$  s.d.) with no significant difference between fetal and adult erythrocytes. The washed erythrocytes were mixed thor-

oughly and haemolyzed (100  $\mu$ l erythrocytes+5 ml distilled water). This haemolysate was used directly for sodium determination and diluted a further 11 times for determination of potassium.

Sodium and potassium were measured in a flame photometer (Unicam). Interference in the potassium analysis by sodium was avoided by the use of a Wratten 35 filter and of a sodium concentration in the potassium standards comparable to that in the samples. No interference by potassium was noted in sodium determinations. Variations in phosphate concentration in the samples were found to have no influence on the potassium or sodium determinations. All sodium and potassium analyses were carried out in quadruplicate. The intracellular concentration of potassium in washed erythrocytes was  $3.6 \pm 0.7\%$  (mean  $\pm$  S.E.M. quadruplicate samples from nine individuals) greater than in unwashed erythrocytes. The measured values of intracellular cation concentrations were therefore corrected with a factor of 3.6% for water lost during washing and centrifugation.

#### Determination of intracellular water

The water content of plasma and whole blood was determined by weighing 100  $\mu$ l samples before and after drying at 100–110°C for 4 hours to constant weight. The intracellular water (in mg/100 mg wet erythrocyte weight) was then calculated according to the formula:

$$\text{Intracellular water} = [100 A - B (100 - H)] / H$$

where  $A$  = water in whole blood,  $B$  = plasma water (in mg water/100 mg whole blood and plasma respectively) and  $H$  = haematocrit value in per cent corrected for trapped plasma. As the centrifugal force in the haematocrit centrifuge used was approximately 10 000  $g$ , a correction figure of 2.5 was used for trapped plasma. (2) Determination of intracellular water was carried out in duplicate. The maximum difference between two measurements was 1.8% of the mean.

All chemicals used were of analytic grade. Double distilled water was used. All glass and polyethylene material was washed in 6 M hydrochloric acid and double distilled water before use. The Student's  $t$  test was used in the statistical analysis.

## RESULTS

#### Sodium and potassium in plasma (Table 1)

In the two groups of women, plasma potassium and sodium concentration was within the normal range (11–17). The potassium concentration in plasma from fetuses, newborns and 1 day-old infants lay close to the upper limit for adults. The difference in plasma potassium concentration between the newborns and their mothers was highly significant but not be-

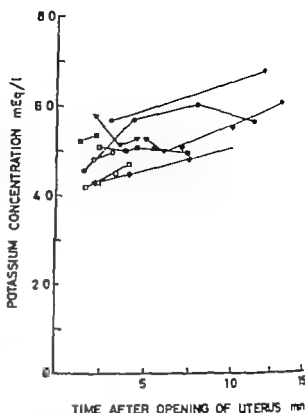


Fig. 1 Potassium concentration in umbilical artery plasma. Successive samples obtained from one to nine mid-term fetuses at different times after opening of uterus.

tween the fetuses and the corresponding mothers.

In order to study whether the interval between uterine incision and collection of the blood influences the measured fetal plasma potassium value, successive samples of blood were obtained from nine fetuses by catheterizing one umbilical artery as soon as possible after opening of the uterus (see Fig. 1). The lines connecting the fetal plasma potassium values obtained in this way were extrapolated to zero time, i.e. to the moment of uterine incision when the maternal blood sample was taken. The first sample from each fetus is possibly subject to errors not likely to influence the following samples, notably a local potassium release from the umbilical vessel wall at the site of catheter insertion. The extrapolation was therefore started at the lowest potassium concentration measured in each series. Fig. 2 illustrates the difference between fetal and maternal plasma potassium concentration.

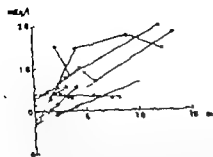


Fig. 2 Difference between fetal and maternal plasma potassium concentration as a function of time after opening of uterus. Same cases as in Fig. 1. Ordinate: Difference between plasma potassium concentration in fetal sample taken at the time indicated and plasma potassium concentration in maternal sample taken at the moment of uterine incision. Abscissa: Minutes of collection of fetal sample minutes after opening of uterus. In all except one case the umbilical cord was clamped before the first sample was obtained. In case □ the cord was not clamped.

extrapolated to zero time. In none of the nine cases did this difference exceed  $\pm 0.5$  mEq/l. It is reasonable to conclude that under normal conditions the potassium concentration in the fetal circulation closely reflects the potassium level in the maternal circulation.

#### Sodium and potassium in erythrocytes (Table 1)

Intracellular potassium did not differ significantly between any two of the five groups of

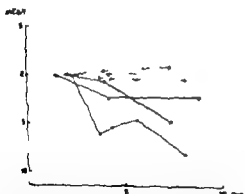


Fig. 3 Changes in intracellular sodium (—) and potassium (---) concentration in fetal erythrocytes as a function of time after opening of uterus. Values from first sample in each series taken as references. Note lowering of intracellular potassium concentration in all three cases.

Table 2 Water in plasma and erythrocytes (RBC) mg H<sub>2</sub>O/mg plasma or wet cell weight  $\pm$  s.e.m.

	Plasma	RBC
I Mid term fetus	96.4 $\pm$ 0.2 n=6	63.8 $\pm$ 0.6 n=6
II Mother (fetus)	91.7 $\pm$ 0.2 n=6	64.2 $\pm$ 0.6 n=6
III Newborn infant	93.1 $\pm$ 0.2 n=5	66.4 $\pm$ 0.4 n=5
IV Mother (new born infant)	91.1 $\pm$ 0.2 n=8	65.3 $\pm$ 0.6 n=8
V 1-day-old infant	92.7 $\pm$ 0.2 n=7	64.4 $\pm$ 0.3 n=6

erythrocytes. Intracellular sodium concentration was lowest in fetal erythrocytes and highest in erythrocytes from newborns and 1-day-old infants. The difference between sodium concentration in fetal and newborn erythrocytes and between newborn and corresponding maternal erythrocytes was highly significant. In three mid term fetuses included in Table 1 intracellular cation content was determined in several blood samples obtained in quick succession from the umbilical artery within 1-9 min after the opening of uterus (Fig. 3). The potassium content of the erythrocytes decreased in all three cases whereas the sodium concentration rose slightly in two of the cases during this period of blood sampling.

#### Water in plasma and erythrocytes (Table 2)

No difference in intracellular water content was found between erythrocytes from any two of the five groups. However the water content of plasma differed between the groups in the following order: mid term fetuses > newborns > one day-old infants > mother (fetuses) = mother (newborns). This is most probably explained by the unequal concentration of protein in the plasma groups (4).

## DISCUSSION

### Methodological considerations

At the analysis of washed erythrocytes it is important to know whether the washing procedure alters the content of the cells. The results of Valberg *et al.* (28) indicate that washing of erythrocytes with magnesium chloride or choline Tris buffer does not interfere

with the determination of the intracellular potassium. The use of washed erythrocytes for the determination of sodium has been criticized (19, 26). However, as the easily exchangeable fraction of intracellular sodium has been found to consist of plasma sodium (see discussion in (2)), the objections to the use of washed erythrocytes for determination of intracellular sodium are no longer valid. Garrahan & Glynn (12) measured a sodium efflux of about 0.6 mmole/litre cell/hour from human adult erythrocytes into choline Ringer solution of the same composition as that used in the present investigation. With the same choline Ringer the sodium influx was found to be 0.2 mmole/litre/hour (12). If we apply these figures to erythrocytes used in the present investigation, a resulting net efflux of sodium of about 0.1 mmole per litre cell volume would occur during the washing procedure used, which lasted for about 10 min.

To judge from determinations of intracellular potassium concentration in the present investigation, cell volume was reduced approximately 3.6% during washing of the cells. It was considered of importance, however, to perform analysis of both intracellular sodium and potassium on identically treated cells; therefore washed cells were used for both types of determination. The procedures used in this investigation for obtaining erythrocytes for sodium and potassium analysis are thus adequate for the present purpose, and the results, after indicated corrections, are comparable with intracellular cation determinations in which trapped plasma is estimated by means of small molecule tracers such as sucrose- $^{14}\text{C}$ .

#### *Sodium and potassium plasma concentrations in fetuses and newborns*

The sodium concentration in plasma from fetuses and newborns was found to lie within the normal adult range (17, 11) in accordance with earlier reports (23, 27, 29, 30). The present investigation also confirms the earlier studies which show a plasma potassium level in fetuses and newborns only slightly above the maternal

level (8, 14, 22, 23, 27). Furthermore, the present results indicate that an increase of plasma potassium concentration in the umbilical artery occurs after even a brief period of disturbance of the umbilical circulation, in agreement with the conclusion reached by James (14). The rapid increase in plasma potassium cannot be ascribed to a potassium efflux from erythrocytes *in vitro* after collection of blood samples, Westin *et al* having shown that in the absence of visible haemolysis the interval between collection of fetal blood and separation of plasma from erythrocytes does not influence the plasma potassium value (29). *In vivo* however, in the present investigation, a rapid release of potassium from the erythrocytes to the plasma occurred after intervention with the umbilical circulation, which may account at least partly for the observed increase in fetal plasma potassium concentration. It is thus probable that the elevated plasma potassium concentration, previously found in human fetuses, premature and newborn infants, do not reflect the normal intrauterine level but rather an effect of blood sampling and/or the influence of labour stress, notably via a decrease in pH (9, 18), causing a shift of potassium from intra- to extracellular fluids. This is supported by the finding that plasma potassium levels decrease during the first 15 min (27) as well as the first 24 hours (1) of extra uterine life.

#### *Sodium and potassium gradients across fetal newborn and maternal red cell membranes*

The potassium and sodium concentration in erythrocytes of the two female groups in the present investigation fell within the normal adult range (3, 7, 10, 28). No significant difference in intracellular potassium concentration was found between any two of the five groups studied. This is in accordance with an earlier report that the potassium concentration in erythrocytes from newborns and their mothers is equal (23).

In the present study intracellular sodium concentration was highest in newborns and 1-day old infants and lowest in mid term fe-

stases. These differences in sodium concentrations remain unexplained. It is of interest that Osterlund too found a higher sodium concentration in erythrocytes from newborns than in red blood cells from their mothers (23). However in his study the absolute values for intracellular sodium concentrations were much higher than in the present investigation which might be explained by the fact that no correction was made for trapped plasma among the erythrocytes.

From the present results it is evident that red blood cells from mid term fetuses, newborns and 1 day old infants have approximately the same capacity to develop ionic gradients across their cell membranes as adult erythrocytes. This conclusion is not compatible with the results of McCance & Widdowson (20). However their findings that the sodium concentration was higher and the potassium concentration lower in fetal than in adult erythrocytes are open to criticism for the following reasons. The number of measurements on human fetal erythrocytes were few, figures for statistical deviations in the material were not given and the sodium concentration reported also for adult human erythrocytes was higher than generally stated. Moreover the authors found a higher water content in fetal than in adult erythrocytes whereas no such difference was found in the present investigation. It should be noted that if any extracellular water is included in the figure for intracellular water it will imply a considerable error in the calculation of intracellular sodium concentration. Each percentage unit of trapped plasma erroneously included in red blood cell volume in cases the figure for intracellular sodium by about 70.

### SUMMARY

Determinations were made of the sodium, potassium and water content of plasma and of washed erythrocytes in blood from human mid term fetuses and their mothers from newborns and their mothers and from 1-day-old infants.

No significant differences in the plasma sodium concentration were found between any two of the five groups of samples. Plasma potassium concentrations in fetal and infant groups were slightly higher than in plasma from their respective mothers (4.91 and 4.68 mEq/l in plasma from fetuses and newborn infants respectively). However plasma potassium in successive samples from the umbilical artery in mid term fetuses rose with time after interference with the umbilical circulation. The difference between mid term fetal and maternal plasma potassium extrapolated backwards to normal intra uterine conditions i.e. to the moment before opening of the uterus was less than  $\pm 0.5$  mEq/l. Factors that are possibly responsible for earlier reported high plasma potassium values in fetuses and newborns are discussed.

The mean intracellular potassium concentration in the five groups of erythrocytes studied varied between 93.5 and 99.5 mEq/kg. No significant difference was found between any two of the five groups. Sodium concentration was higher in erythrocytes from newborn infants and 1 day old infants than in those from the other three groups.

It is concluded that the fetal plasma potassium concentration at mid term closely reflects the maternal level and that red blood cells from human fetuses at mid term and at term are capable of generating ionic gradients across the cell membrane of the same magnitudes as the adult red blood cells.

### ACKNOWLEDGEMENTS

The authors wish to thank Margaretha Olsson, Maria Wroblewski and Karin Jonsson for skilful technical assistance. This work was supported by grants from the Medical Faculty University of Lund and the Swedish National Association against Heart and Chest Diseases.

### REFERENCES

1. Acharya, P. T. & Payne, W. W. Blood chemistry of normal full term infants in the first 48 hours of life. *Arch Dis Child* 40: 430, 1965.

- Beilin L J Knight G J Munro Faure A D & Andersson J The measurement of sodium concentration in human red blood cells *J Gen Physiol* 50 61 1966
- 3 — The sodium potassium and water contents of red blood cells of healthy human adults *J Clin Invest* 45 11 1966
- 4 Bergstrand C G & Czar B Paper electrophoretic study of human fetal serum proteins with demonstration of new protein fraction *Scand J Clin Lab Invest* 9 277 1957
- 5 Bratteby L E Garby L Groth T Schneider W & Widman B Studies on Erythrokinetics in Infancy XIII The mean life span frequency function of red blood cells formed during foetal life *Acta Paediat Scand* 57 311 1966
- 6 Crowley J Wray P & Jones J Human fetal erythrocyte and plasma lipids *J Clin Invest* 44 989 1965
- 7 Czeizler J W Ullman T D Ullman I & Bar Kochbi Z Determination of the red blood cell content of water sodium and potassium in normal subjects *J Lab Clin Med* 61 973 1963
- 8 Earle D P Baldwin H & Hirsch H The plasma potassium level in the newborn *Proc Soc Exp Biol Med* 76 756 1951
- 9 Engstrom L Karlberg P Rooth G & Tunell R The onset of respiration A study of respiration and changes in blood gases and acid base balance Association for the Aid of Crippled Children New York 1966
- 10 Funder J & Wieth J O Potassium sodium and water in normal human red blood cells *Scand J Clin Lab Invest* 18 167 1966
- 11 Garrot P O Laurell C B & Norström B *Apm pendium i klinisk kemi* Studentlitteratur Lund 1968
- 12 Gattahon P J & Glynn I M The behaviour of the sodium pump in red cells in the absence of external potassium *J Physiol* 192 159 1967
- 13 Hollán S R Székely J G Brewer J H Medgyesi G & Soter V N Differences between the structure and function of human adult and foetal erythrocytes *Folia Haematol* 90 126 1968
- 14 James L S Acidosis of the newborn and its relation to birth asphyxia *Acta Paediat Scand* 49 17 1960 Suppl 122
- 15 Keitel H G The concentration of potassium in the plasma *Amer J Dis Child* 97 593 1959
- 16 Kerpel-Fronius E Nagy L & Bata G Kalidmoe et potassium treuslure du nouveau né *Arch Franc Pédiat* 19 386 1962
- 17 Karschbaum T H & DeHaven J C Maternal and fetal blood constituents *In N B Ascoli (ed) Biology of gestation vol II The fetus and neonate* Academic Press New York and London 1968
- 18 Koch G & Wendel H Adjustment of arterial blood gases and acid base balance in the normal newborn infant during the first week of life *Biol Neonat* 12 136 1968
- 19 Muzela M & Remington M Cation exchange of human erythrocytes *J Physiol* 145 641 1959
- 20 McCance R A & Widdowson E M The effect of development anemia and undernutrition on the composition of the erythrocytes *Clin Sci* 15 409 1956
- 21 Newman R L Serum electrolytes in prematurity and puerperium *Obst Gyn* 18 1 1957
- 22 Overman R R Etteldorf J N Pars J N Horn G B *Pediatrics* 7 565 1951
- 23 Osterlund K A comparative investigation of the concentration of certain electrolytes in maternal and cord blood *Ann Paediat Fenn* 1 Suppl 42 1955
- 24 Spolin H The resistance of red cells to water *Acta Paediat Scand* 43 Suppl 98 1 1954
- 25 Smith C H *Blood diseases of infancy and childhood* C V Mosby St Louis 1960 p 1
- 26 Streif G M Sodium and calcium content of erythrocytes *J Biol Chem* 179 661 1959
- 27 Thilme B Electrolyte and acid base balance in fetal and maternal blood *Acta Obstet Gyn Scand* 45 Suppl 9 7 1967
- 28 Vilberg L S Holt J M Paulson E & Sævi J Spectrochemical analysis of sodium potassium calcium magnesium copper and zinc in normal erythrocytes *J Clin Invest* 44 379 1966
- 29 Westin B Kärner I H Lind J Nyberg R Teger-Nilsson A C Some constituents of umbilical venous blood of previsible human fetuses *Acta Paediat Scand* 49 609 1959
- 30 Widdowson E M & McCance R A The effect of development on the composition of the serum and extracellular fluids *Clin Sci* 15 6 1956
- 31 Widdowson E M Growth and Composition of the Fetus and Newborn *In N S Ascoli (ed) Biology of Gestation vol II The fetus and neonate* Academic Press New York and London 1968 p 1
- 32 Zipursky A LaRue T & Ismail L G The in vitro metabolism of erythrocytes from newborn infants *Can J Biochem Physiol* 38 727 1960

Submitted June 5 1969

Accepted Sept 24 1969

(B B) Dept of Pharmacology  
Solvegatan 10  
223 62 Lund  
Sweden

**Key words** Human fetus newborn infants sodium and potassium in plasma sodium and potassium in erythrocytes

## SOMATIC DEVIATIONS IN NEWBORN AND OLDER MONGOLOID CHILDREN

### *A Follow up Investigation*

BERTIL HALL

*From the Institute of Genetics and the Department of Paediatrics  
University of Lund, Lund, Sweden*

We know that the most characteristic features (the cardinal signs) of newborn (1) and older mongoloid children (2) are not identical but it is not clear how and when the various signs change. The approach to this problem can best be made by investigating a random material of mongoloid children. This investigation should then be followed up for many years. Because many mongoloid children die in infancy the material must be collected during the newborn period. No such investigation has been reported earlier. A limiting factor has been the difficulty in diagnosing mongolism in some of the mongoloid newborns which mainly depends on the large variation that exists. This limiting factor vanished when the development of the cytogenetic analysis increased the diagnostic possibilities.

This article gives an account of a material of newborn mongoloid children who were investigated several times up to the age of 6 years.

### MATERIAL

From July 1 1961 to June 30 1962 all newborn suspected mongoloids in southern Sweden (Göteborg, Malmö, Halmstad and Västerås) were examined by the author (1). During this period 11 083 children were born of which 43 were suspected as being mongoloid. Renewed clinical examinations and chromosome analyses could exclude the diagnosis of mongolism in five instances. Thus, 38 had an extra G chromosome. The found frequency 0.15% (1/660)

agrees with earlier investigations carried out in Europe (3). By various control methods I could be certain that all mongoloids born during the relevant period had been reported. Of the five who were not mongoloids one had an eyelid defect (microblepharism), one showed signs of thalidomide injury with among other things seriously dysplastic ears and one had a flat facial profile and an abundance of neck skin. The other two infants were severely dysplastic and later showed signs of mental retardation.

### MORTALITY AND CARDIAC MALFORMATIONS

During the first 6-7 years the material was reduced by 16 children, 15 of whom died before the age of 6 months. Autopsies were performed on 10 of these, all except one being performed at a pathological institution. All 10 were found to have had congenital vicia, one had an atrial septal defect, two had ostium atriocentricular commissure and the remaining seven had an uncomplicated ventricular septal defect. In the newborn period a cardiac X ray was taken in nine of the children who were autopsied. A deviating heart configuration was found in seven of them while in two the findings were uncertain. As a comparison it can be mentioned that 18 mongoloid children without cardiac symptoms in the newborn period or later were also examined by X ray in the newborn period. The heart configuration was considered normal in 13 cases, uncertain in two and abnormal in three.

Another two of the deceased children had shown signs of cardiac malformation, *inter alia* harsh systolic murmur and abnormal heart configuration during the X ray examination. Of the 15 deceased in fact 13 thus had a congenital heart defect.

These 13 children had displayed the following signs of cardiac malformation. Four of them had a harsh systolic murmur at newborn whereas in two others the murmur did not appear until the age of 14 days.



In one child who also had cyanosis only a weak systolic murmur could be detected. This however disappeared after two months. Three other children manifested cyanosis and dyspnoea following exertion, but otherwise had a normal physical finding whereas another infant had only an accentuated second sound. One child died 10 hours after birth.

A new X-ray examination of the heart supplemented by an EKG was carried out at the age of 12 months in four subjects because the examination in the newborn period could not exclude cardiac malformation. Here obvious signs of a congenital vitium were found in two children and it was not until they were 12 months old that the systolic murmur was heard. Both children are still living at 6 years of age. One of them has suffered several severe episodes of illness with high fever and signs of heart compensation. Thus 14 (12+2) of the 38 children (37%) in this mongoloid material have been found to have a cardiac malformation. Only three of those who died before reaching the age of one year had no clinical signs of congenital heart malformation. The cardiac X-rays were normal and no heart murmur could be detected. However it was noted that these children too became breathless in connection with feeding.

An infection usually bronchopneumonia or gastroenteritis was a contributory cause of death in most of the children. In some cases only fever was reported. The others who died without signs of infection had severe cardiac malformations (four cases) and duodenal atresia plus cardiac malformation (one case). Death also often occurred quickly: two died in their homes and three on the way to the hospital or just after arrival.

After the first year only one of the children had died. The cause of death was leukemia which is a known complication of mongolism. Thus 22 of the original 38 mongoloid children are still alive after 11 years.

## METHOD

The 22 children had first been examined as newborn and then again at the ages of about 1, 3 and 6 years by the author. (One child was examined by another person at the 3 and 6 year controls.) Various external mongoloid signs were appraised. Head circumference, cephalic index, length, weight and chest circumference were also recorded. Repeated X-rays of the skeleton could also be performed in some of the children. The children were also appraised according to Vineland's Social Maturity Scale. Only certain parts of this follow up investigation will be commented on in this article.

## THE NURSING SITUATION OF THE MONGOLOIDS

Since early infancy three of the 22 children have been cared for outside of their own homes. One

child has been in a nursing home since the age of 4 years but visits its own home during holidays. Another child was placed in a nursing home at the age of 6 years. Of the 22 children 17 are still at 6 years of age in their own homes.

## RESULTS

Not less than 20 of the mothers of the mongoloid children were 35 years old or more at the time of the birth of their child. The average age is high: 34.7 years. Seven of the children were premature. The average birth weight was 3060 g, i.e. 4 hg less than the average normal newborn. This can only partly be explained by the pregnancy period usually being one week shorter than normal.

### *Various signs in the newborn period*

The general phenotype of the newborn mongoloid children was described in an earlier work (1). Hence only a short description of the various signs of the newborn mongoloid will be given here.

The facial expression of the newborn is often quite characteristic: small mouth, a nose oblique palpebral fissures and the expressionless mien. The facial appearance of the mongoloids however is far from identical. One of the signs first noticed is a pronounced pallor. Their passivity is also striking. They lack the spontaneous motility of the normal child. The Moro reflex is usually absent. Muscle hypotonia is common and mainly noticed by the one who nurses the child. The skin is pasty, the neck skin being particularly abundant. Hyperflexibility is another obvious sign. The children cry seldom and then weakly. The unusual crying often creates suspicion in the mother that all is not well.

The most usual eye signs are iris spots and the oblique palpebral fissures. The newborns have no true epicanthus but their medial eye angle is blunt and not normally pointed. Considerable information can be obtained by examining the profile, which is flat. The nasbridge especially is hardly developed. The head is round and lacks the powerful occipital

characterizes normal newborn its circumference is small (average value = 33.0 cm). The ear is small and lacks distinct contours. It does not have the normal oval shape but is round or almost square. Usually only the upper part of the helix is folded. The mouth similar to the nose is small and the corners usually droop. The tongue often protrudes although this is not so obvious. In scarcely half of the children the palm of the hand shows a four finger line but in almost all the palms are poorly differentiated with many small furrows and indistinct main furrows. The short broad mongoloid hand is difficult to appraise in the newborns because they practically always have their hands clenched. The nails may be hyperconvex. There is often a considerable space between the first and second toes. The X-ray examinations showed that more than half of the children had a dysplastic middle phalanx of the little finger (see Fig. 1) and most had a broad low pelvis. This deviating pelvic form can also be expressed by the use of an index Cuffey's index which is half the sum of the acetabular and iliac angles. The average value for the mongoloid children was 69 which was 10 less than for normal controls.



Fig. 1 Schematic drawings of the skeleton of the fifth finger from the same mongoloid patient as assessed as a newborn and on three other occasions showing a normalisation of the dysplastic middle phalanx (arrow).

after the age of 12 months but later disappears almost completely. Only two of the 6-year olds had a protruding tongue. The oblique palpebral fissures are very characteristic of the mongoloids; they become more obvious when

#### Signs that had diminished or disappeared<sup>1</sup> (cf. also Fig. 2)

At the follow up investigation the following signs showed a reduced strength of frequency. The obvious pallor of the newborns was still present at the age of 6 years but was too weak to be of diagnostic value. The newborns had a weak, characteristic cry but all screamed normally at 6 years of age. Another characteristic sign as the newborn the abundant pasty neck skin also tends to disappear; it was observed in only three children at the 6-year investigation. The skin also seemed less pasty at this age. There were less children whose tongues protruded. This sign is more noticeable when the children begin to suck their tongues.

The frequencies are given only for the children who survived infancy.

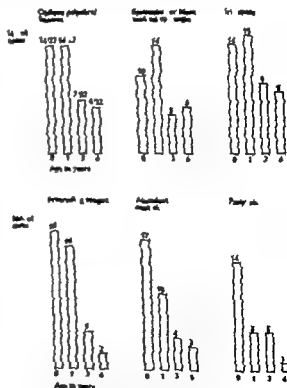


Fig. 2 The frequencies are given for some mongoloid signs in a mongoloid material (25 cases) which have been examined since the newborn period. Before the last two investigations the material was reduced by one patient.



Fig 3 Age of walking

the child cries. Among the newborn, 14 out of 23 had this sign but the figure was reduced to six at the age of 6 years. Another eye sign the iris spots, also showed the same tendency to disappear and was found in fewer 6 year olds.

The epicanthal fold is not developed in the newborn. When the children were 12 months old however a distinct epicanthus was observed in 14 out of 23 children but later this again reduced in frequency. At 6 years of age six children had this less valuable sign which is also common in normal children. Muscle hypotonia is another feature that disappears. Obvious hypotonia was noted in 13 of the newborn and moderate hypotonia in only five of the 6 year olds. The hyperconvex nails of the newborn had become normal at the 6 year control. In some children with a dysplastic middle phalanx of the little finger a tendency for normalization was noted at the follow up investigations even though one or another of the middle phalanges were not wholly normal. The most obvious example is shown in Fig. 1. An X ray examination of the hand skeleton could be made in only 15 of the 6 year olds.

Snuffly breathing is another feature that characterizes the mongoloid children. During the first year of infancy children with snuffly breathing increased from 5 to 10. Thereafter the breathing improved and at 6 years of age all breathed normally. The most natural explanation is that the children's normal susceptibility to infection decreased considerably from the age of 4 years on. This applies also to the tendency for blepharitis. In 6 years of age suffer a slight attack of blepharitis with a severe cold.

### Signs that had not changed

The frequency of hyperflexible children is not changed up to the age of 6 years although the flexibility in the joints was more pronounced in the newborn. Equally many of the older children seem also to have a flat face profile. The external ear had not changed shape to an extent worth mentioning. However the ear was no longer flabby as in the newborn instead its contours were more firm. Most of the mongoloids, both as newborn and as 6 year olds had a short, broad hand. The foal finger line occurred with the same frequency at the different ages.

### Signs that had developed or had become strengthened

The 6 year-old children often have dry skin and their cheeks have a tendency toward redness especially after remaining out-of-door. The round head shape of the newborn changed and the 6 year old children are instead characterized by a flat occiput (16 out of 22 children). Short stature is more obvious in the older children. The average value for the 6 year old mongoloids was 108 cm. The mongoloids grow more slowly than normal children. It can also be noted in the mongoloids that the thorax often has a deformed form. It is usually broad and has a slight depression at the lower part of the sternum. In the older mongoloid children it is more noticeable that the foot is short, clumsy and somewhat splayed. The space between the first and second toes is not only large but the big toe shows a tendency to point medially. In the newborn only the large space between the first and second toes was noted. The difference is partly a result of the load that the foot is subjected to. The bent little fingers are also more conspicuous in the older children. In some of the children the tongue begins to develop fissures. The pelvis becomes increasingly dysplastic. The mean value for Caffey's index has thus decreased by 21 since the newborn period.

### *Some observations on the functions of the 6 year old children*

Only one child was so retarded that even at 6 years of age it could not understand what was said to it. One girl impressed us by being able to speak almost normally. Most of the children spoke in a manner that could be understood only by their relatives but six of them could compose quite understandable sentences. Almost as many (five) could not speak but only made special sounds. Most of them were somewhat hoarse.

No less than 17 out of the 22 children could feed themselves. Somewhat more—16—could get water to drink and 13 could ask for permission to go to the toilet. Three more could be managed by careful attention and eight of them had nocturnal enuresis. Fourteen children were unable to descend a staircase normally. The large variation in development in this material can be demonstrated by the debut of walking which is shown in Fig. 3. Two of the children who did not walk until after the age of 4 years deviated also in other ways from the other children. One was also poorly developed in other respects, i.e. lacked the faculty of speech and displayed much stereotypy whereas the other child had congenital cataracts and concomitant blindness.

### DISCUSSION

The material consists of all the mongoloid children born in a large geographical area therefore despite its smallness it should be representative of mongoloids in general. Comparisons were made with larger materials especially concerning data that could be extracted from case records and autopsy reports and have also shown good agreement. Forsman & Almqvist (4) found in a larger Swedish material that the mothers' average age at the birth of the mongoloid child was 35.4 years; this does not differ appreciably from the average in the present material, 34.7 years. The debut of walking was compared with the results in the material of Levinson *et al.* (5).

There is a slight discrepancy between the materials. More mongoloids in their material began to walk at 2 years of age while in the present material the debut of walking usually occurred at the age of 2½ years. There is however a wide distribution pattern in both materials. Rowe & Uchida (6) investigated the most representative mongoloid material; this however is not altogether free from objections. Their intention was to determine the cardiac malformation frequency. They found that 40% of the mongoloids had cardiac malformations; this corresponds to 37% in the present material. Earlier it was maintained that A-V communis is the dominant defect in mongolism. Berg *et al.* (7) however in a large autopsy material could show that the ventricular septal defect is the most common finding in mongoloids. A-V communis is more common in mongoloids than in normals. Also in the present material the ventricular septal defects dominated and A-V communis occurred in no less than 2 of the 10 autopsied. Eken (8) showed that 9 out of 12 mongoloid children with cardiac malformations died before 2 years of age whereas only 3 out of 19 children without congenital heart disease died during the same period. As early as 1940 Evans (9) pointed out that mongoloids with cardiac malformations usually die between the ages of two and five months. In the present material most of the children with a congenital cardiac vitium also died before the age of 5 months (10 out of 14) whereas only 3 out of the other 24 children died during the first two years of life. The comparisons thus argue that the present material is representative of mongoloids in general.

The prognosis for the mongoloids with congenital heart malformations is thus very poor. Most die during the first year of infancy. This cardiac malformation occurs also at such a high frequency that a cardiological investigation (with *inter alia* X rays) of the newborn mongoloid children is to the greatest extent justified.

It is usually maintained that the clinical



Fig. 3 Age of walking

the child cries. Among the newborn 14 out of 23 had this sign but the figure was reduced to six at the age of 6 years. Another eye sign the iris spots also showed the same tendency to disappear and was found in fewer 6 year olds.

The epicanthal fold is not developed in the newborn. When the children were 12 months old however, a distinct epicanthus was observed in 14 out of 23 children but later this again reduced in frequency. At 6 years of age six children had this less valuable sign which is also common in normal children. Muscle hypotonia is another feature that disappears. Obvious hypotonia was noted in 13 of the newborn and moderate hypotonia in only five of the 6 year olds. The hyperconvex nails of the newborn had become normal at the 6 year control. In some children with a dysplastic middle phalanx of the little finger a tendency for normalization was noted at the follow up investigations even though one or another of the middle phalanges were not wholly normal. The most obvious example is shown in Fig. 1. An X ray examination of the hand skeleton could be made in only 15 of the 6 year olds.

Snuffly breathing is another feature that characterizes the mongoloid children. During the first year of infancy children with snuffly breathing increased from 5 to 10. Thereafter the breathing improved and at 6 years of age all breathed normally. The most natural explanation is that the children's normal susceptibility to infection decreased considerably from the age of 4 years on. This applies also to the tendency for blepharitis. In 6 years of age suffer a slight attack of blepharitis with a severe cold.

### Signs that had not changed

The frequency of hyperflexible children had not changed up to the age of 6 years although the flexibility in the joints was more pronounced in the newborn. Equally many of the older children seem also to have a flat facial profile. The external ear had not changed in shape to an extent worth mentioning. However the ear was no longer flabby as in the newborn instead its contours were more firm. Most of the mongoloids, both as newborn and as 6 year olds, had a short broad hand. The four finger line occurred with the same frequency at the different ages.

### Signs that had developed or had become strengthened

The 6 year-old children often have dry skin and their cheeks have a tendency toward redness especially after remaining out-of-doors. The round head shape of the newborn is changed and the 6 year old children are instead characterized by a flat occiput (16 out of 22 children). Short stature is more obvious in the older children. The average value for the 6 year old mongoloids was 108 cm. The mongoloids grow more slowly than normal children. It can also be noted in the mongoloids that the thorax often has a deviation form it is usually broad and has a slight depression at the lower part of the sternum. In the older mongoloid children it is more noticeable that the foot is short clumsy and somewhat splayed. The space between the first and second toes is not only large but the big toe shows a tendency to point medially. In the newborn only the large space between the first and second toes was noted. The difference is partly a result of the fact that the foot is subjected to. The bent little fingers are also more conspicuous in the older children. In some of the children the tongue begins to develop fissures. The pelvis becomes increasingly dysplastic. The mean value for Coffey's index had thus decreased by 21 since the newborn period.

### Some observations on the functions of the 6 year-old children

Only one child was so retarded that even at 6 years of age it could not understand what was said to it. One girl impressed us by being able to speak almost normally. Most of the children spoke in a manner that could be understood only by their relatives, but six of them could compose quite understandable sentences. Almost as many (five) could not speak, but only made special sounds. Most of them were somewhat hoarse.

No less than 13 out of the 22 children could feed themselves. Somewhat more—16—could get water to drink, and 13 could ask for permission to go to the toilet. Three more could be managed by careful attention and eight of them had nocturnal enuresis. Fourteen children were unable to descend a staircase normally. The large variation in development in this material can be demonstrated by the debut of walking, which is shown in Fig. 3. Two of the children who did not walk until after the age of 4 years deviated also in other ways from the other children. One was also poorly developed in other respects, i.e. lacked the faculty of speech and displayed much stereotypy, whereas the other child had congenital cataracts and concomitant blindness.

### DISCUSSION

The material consists of all the mongoloid children born in a large geographical area, therefore, despite its smallness, it should be representative of mongoloids in general. Comparisons were made with larger materials especially concerning data that could be extracted from case records and autopsy reports and have also shown good agreement. Forsman & Åkesson (4) found in a larger Swedish material that the mothers' average age at the birth of the mongoloid child was 35.4 years; this does not differ appreciably from the average age in the present material, 34.7 years. The debut of walking was compared with the results in the material of Levinson *et al.* (3).

There is a slight discrepancy between the materials. More mongoloids in their material began to walk at 2 years of age, while in the present material the debut of walking usually occurred at the age of 2½ years. There is, however, a wide distribution pattern in both materials. Rowe & Uchida (6) investigated the most representative mongoloid material; this, however, is not altogether free from objections. Their intention was to determine the cardiac malformation frequency. They found that 40% of the mongoloids had cardiac malformations; this corresponds to 37% in the present material. Earlier it was maintained that A-V communis is the dominant defect in mongoloids. Berg *et al.* (7) however in a large autopsy material could show that the ventricular septal defect is the most common finding in mongoloids. A-V communis is more common in mongoloids than in normals. Also in the present material the ventricular septal defects dominated and A-V communis occurred in no less than 2 of the 10 autopsied. Esen (8) showed that 9 out of 12 mongoloid children with cardiac malformations died before 2 years of age, whereas only 3 out of 19 children without congenital heart disease died during the same period. As early as 1930 Evans (9) pointed out that mongoloids with cardiac malformations usually die between the ages of two and five months. In the present material most of the children with a congenital cardiac vitium also died before the age of 5 months (10 out of 14), whereas only 3 out of the other 24 children died during the first two years of life. The comparisons thus argue that the present material is representative of mongoloids in general.

The prognosis for the mongoloids with congenital heart malformations is thus very poor. Most die during the first year of infancy. This cardiac malformation occurs also at such a high frequency that a cardiological investigation (with *inter alia* X-rays) of the newborn mongoloid children is to the greatest extent justified.

It is usually maintained that the clinical

diagnosis of mongolism is most difficult to make during the newborn period. This can be interpreted in such a way that the mongoloid phenotype is more pronounced as the child grows older. The alternative explanation is that the lack of distinct signs of mental retardation in the newborn contributes to the diagnosis being so difficult to make. According to the present investigation the latter alternative is the most probable one. Many of the signs in the newborn are either diminished or have disappeared whereas only a few signs have been strengthened or added. Thus the mongoloid phenotype is most pronounced in the newborn child. Many of the signs involved could only be appraised subjectively. One of the few mongoloid signs that can be measured is the head circumference. An earlier investigation (10) showed that the mongoloids with the smallest head circumference as newborn grew most rapidly with respect to head circumference which is also a form of normalization. On the whole however mongoloids grow much more slowly than normal children. At 6 years of age they are of conspicuously short stature and the picture is dominated by mental retardation especially their poor speech.

#### ACKNOWLEDGEMENT

The author is indebted to *Expressens* prenatal research fund and to the Swedish May Flower Foundation for economic support of the investigation.

#### REFERENCES

1. Hall B. Mongolism in newborns. A clinical and cytogenetic study. *Acta Paediat Scand Suppl* 154 1964.
2. Öster J. *Mongolism*. Danish Science Press Ltd. Copenhagen 1953.
3. Penrose L. S. & Smith G. F. *Down's Anomaly*. J. & A. Churchill Ltd. London 1966.
4. Forsman H. & Akesson H. O. Personal communication.
5. Levinson A., Friedman A. & Stamps F. Variability of mongolism. *Pediatrics* 16 43 1955.
6. Rowe R. D. & Uchida I. A. Cardiac malformations in mongolism. *Amer J Med* 31 726 1961.
7. Berg J. M., Crome L. & France N. E. Congenital cardiac malformations in mongolism. *Brit Heart J* 22 331 1960.
8. Eken F. M. Congenital heart malformations in mongolism with special reference to osium atri-ventricularis commune. *Arch Pediat* 74 143 1957.
9. Evans P. R. Cardiac anomalies in mongolism. *Brit Heart J* 12 258 1950.
10. Hall B. Follow up investigation of newborn mongoloids with respect to growth retardation. *Hereditas* 56 99 1966.

Submitted July 31 1969

Accepted Sept. 21 1969

Institute of Genetics  
Lunds universitet  
223 62 Lund  
Sweden

**Key words:** Mongolism. Down's syndrome. follow up investigation.

## ACID PHOSPHATASE ACTIVITY IN THE SYNOVIAL FLUID OF CHILDREN WITH JUVENILE RHEUMATOID ARTHRITIS

B. BECKMAN, L. BECKMAN, L. GÖTHEFORS and R. LEMPERG

*From the Departments of Clinical Bacteriology, Orthopedic Surgery and of Paediatrics, University of Umeå, Umeå, Sweden*

The diagnosis of juvenile rheumatoid arthritis (JRA) is at present not easy to establish at an early stage of the disease. The serological reactions are usually negative especially in monarticular cases (2-4). The diagnosis is therefore usually arrived at by the exclusion of other diseases such as tuberculous bacterial arthritis and ulcerative colitis or by the subsequent involvement of additional joints.

In a previous study of synovial fluid (1) the authors found a significantly increased acid phosphatase activity in adult patients with rheumatoid arthritis in comparison with patients with non-inflammatory joint disorders. Furthermore a number of patients with monarticular synovitis and high acid phosphatase activity but negative serological reactions were found to develop definite rheumatoid arthritis within 1 year.

It was therefore considered of interest to examine children with different joint effusions with the aim of assessing whether determinations of acid phosphatase activity in the synovial fluids may help to ascertain early diagnosis of a rheumatoid joint disorder.

## MATERIAL AND METHODS

From 1 children with effusions in one or more joints synovial fluid was obtained by joint puncture. Only cases which have been followed up for at least half a year after joint fluid examination are included in this study. The diagnoses at the time of joint fluid aspiration were in these patients as follows. (See also Table 1).

In 5 cases with engagements of more than one joint

the diagnosis of JRA was made according to the criteria recommended by Edström (3) after exclusion of other diseases. These patients had one knee and in addition one or two major joints engaged.

Seven patients had only one joint affected. In two of them the diagnosis of JRA was made according to the criteria recommended by Canady et al. (2). Both had a chronic synovitis of one knee joint with a duration of more than 4 months. Of the remaining 5 patients 4 had a monarticular joint effusion of short duration and unknown aetiology in either the knee or the hip joint. One patient had persisting effusion after a bacterial arthritis confirmed by bacterial culture from the synovial fluid 1 month earlier.

In all patients standard tests for rheumatoid factors: roentgenograms of the affected joints, chest film, L.E. cell test, ascorbic acid count and paper electrophoresis of the serum proteins were made.

All samples of synovial fluid were subjected to routine bacteriological examination. In 7 cases total and differential white cell counts of the synovial fluid were performed. Synovial biopsies were obtained in 6 cases in connection with synovectomy performed at different intervals after joint fluid examination. Determinations of acid phosphatase activity were made in 11 as naphthyl phosphate in substrate (1).

## RESULTS

None of the patients had a positive serological reaction for rheumatoid arthritis. The results of the chest film, L.E. cell tests, blood cell counts and serum protein electrophoresis did not indicate the presence of diseases other than rheumatoid arthritis. The roentgenological findings were normal or compatible with JRA with the exception of one patient (no. 9). All patients operated on with synovectomy showed at inspection of the joint a picture of extensive inflammation of the tunica synovialis. The



diagnosis of mongolism is most difficult to make during the newborn period. This can be interpreted in such a way that the mongoloid phenotype is more pronounced as the child grows older. The alternative explanation is that the lack of distinct signs of mental retardation in the newborn contributes to the diagnosis being so difficult to make. According to the present investigation the latter alternative is the most probable one. Many of the signs in the newborn are either diminished or have disappeared, whereas only a few signs have been strengthened or added. Thus the mongoloid phenotype is most pronounced in the newborn child. Many of the signs involved could only be appraised subjectively. One of the few mongoloid signs that can be measured is the head circumference. An earlier investigation (10) showed that the mongoloids with the smallest head circumference as newborn grew most rapidly with respect to head circumference which is also a form of normalization. On the whole however mongoloids grow much more slowly than normal children. At 6 years of age they are of conspicuously short stature and the picture is dominated by mental retardation especially their poor speech.

#### ACKNOWLEDGEMENT

The author is indebted to *Expressens* prenatal research fund and to the Swedish May Flower Foundation for economic support of the investigation.

#### REFERENCES

- 1 Hall B. Mongolism in newborns. A clinical and cytogenetic study. *Acta Paediat Scand Suppl.* 154 1964.
- 2 Øster J. *Mongolism*. Danish Science Press Ltd Copenhagen 1953.
- 3 Penrose L. S. & Smith G. F. *Down's Anomaly*. J. & A. Churchill Ltd London 1966.
- 4 Forsman H. & Åkesson H. O. Personal communication.
- 5 Levinson A., Friedman, A. & Stamps F. Variability of mongolism. *Pediatrics* 16 43 1955.
- 6 Rowe R. D. & Uchida I. A. Cardiac malformations in mongolism. *Amer J Med* 31 726 1961.
- 7 Berg J. M., Crume, L. & France N. E. Congenital cardiac malformations in mongolism. *Brit Heart J* 22 331 1960.
- 8 Esen F. M. Congenital heart malformations in mongolism with special reference to osseous and ventricular commune. *Arch Paediat* 74 743 1957.
- 9 Evans P. R. Cardiac anomalies in mongolism. *Brit Heart J* 12 258 1950.
- 10 Hall B. Follow up investigation of newborn mongoloids with respect to growth retardation. *Hereditas* 56 99 1966.

Submitted July 31 1969

Accepted Sept. 21 1969

Institute of Genetics  
Lunds universitet  
223 62 Lund  
Sweden

*Key words:* Mongolism. Down's syndrome. follow up investigation.

## ACID PHOSPHATASE ACTIVITY IN THE SYNOVIAL FLUID OF CHILDREN WITH JUVENILE RHEUMATOID ARTHRITIS

G. BECKMAN, L. BECKMAN, L. GÖTHEFORS and R. LEMPERO

From the Departments of Clinical Bacteriology, Orthopedic Surgery and of Pediatrics, University of Umeå, Umeå, Sweden

The diagnosis of juvenile rheumatoid arthritis (JRA) is at present not easy to establish at an early stage of the disease. The serological reactions are usually negative, especially in monarticular cases (2, 4). The diagnosis is therefore usually arrived at by the exclusion of other diseases such as tuberculous bacterial arthritis and ulcerative colitis or by the subsequent involvement of additional joints.

In a previous study of synovial fluid (1) the authors found a significantly increased acid phosphatase activity in adult patients with rheumatoid arthritis in comparison with patients with non-inflammatory joint disorders. Furthermore a number of patients with monarticular synovitis and high acid phosphatase activity but negative serological reactions were found to develop definite rheumatoid arthritis within 1 year.

It was therefore considered of interest to examine children with different joint effusions with the aim of assessing whether determinations of acid phosphatase activity in the synovial fluids may help to ascertain early diagnosis of a rheumatoid joint disorder.

### MATERIAL AND METHODS

From 11 children with effusions in one or more joints synovial fluid was obtained by joint puncture. Only cases which have been followed up for at least half a year after joint fluid examination are included in this study. The diagnoses at the time of joint fluid aspiration were in these patients as follows (See also Table 1).

In 5 cases with engagements of more than one joint

the diagnosis of JRA was made according to the criteria recommended by Edsstrom (3) after exclusion of other diseases. These patients had one knee and in addition one or two major joints engaged.

Seven patients had only one joint affected. In two of them the diagnosis of JRA was made according to the criteria recommended by Cassidy *et al.* (2). Both had a chronic synovitis of one knee joint with a duration of more than 4 months. Of the remaining 5 patients 4 had a monoarticular joint effusion of short duration and unknown etiology in either the knee or the hip joint. One patient had persisting effusion after a bacterial arthritis confirmed by bacterial culture from the synovial fluid 1 month earlier.

In all patients standard tests for rheumatoid factors, roentgenograms of the affected joints, chest film, L.E. cell test, complete blood count and paper electrophoresis of the serum proteins were made.

All samples of synovial fluid were subjected to routine bacteriological examination. In 7 cases total and differential white cell counts of the synovial fluid were performed. Synovial biopsies were obtained in 6 cases in connection with synovectomy performed at different intervals after joint fluid examination. Determinations of acid phosphatase activity were made with a naphthyl phosphate as substrate (1).

### RESULTS

None of the patients had a positive serological reaction for rheumatoid arthritis. The results of the chest film, L.E. cell tests, blood cell counts and serum protein electrophoresis did not indicate the presence of diseases other than rheumatoid arthritis. The roentgenological findings were normal or compatible with JRA with the exception of one patient (no. 9). All patients operated on with synovectomy showed at inspection of the joint a picture of extensive inflammation of the tunica synovialis. The

Table 1 *Clinical data and acid phosphatase activity*

Activity expressed in micromols of  $\alpha$  naphthyl phosphate hydrolyzed per ml fluid per hour. The clinical diagnosis and duration of illness refers to the time of joint fluid analysis

Case no	Age (years)	Clinical diagnosis	Duration of illness	No of joints involved	Acid phosphatase	Remarks
1	8	JRA	7 years	2	1.13	
2	8	JRA	7 years	3	4.12	
Same patient 1 month after synovectomy					1.39	
3	8	JRA	3 years	2	2.52	
4	10	JRA	8 years	2	3.09	
Same patient 2 months after synovectomy					2.12	
5	13	JRA	2 years	3	1.06	
6	13	JRA	1 year	1	3.97	No further joint engaged after 2 years
Same patient 9 months after synovectomy					0.12	
7	14	JRA	9 months	1	3.97	One year later still joint effusion no further joint engaged
8	1	Monarticular synovitis	1 month	1	1.69	Op. one year later. Diagn. JRA confirmed by synovial biopsy
9	4	Monarticular synovitis (bacterial osteo-arthritis?)	6 weeks	1	3.93	After 9 months still signs of synovitis. No other joint engaged. Diagnosis still uncertain
10	12	Monarticular synovitis	5 weeks	1	1.47	Op. 5 months later. No further joint engaged after 9 months
11	4	Transient arthritis of the hip	1 day	1	0.30	Recovered no further joint engaged after 1 year
12	7	Bacterial arthritis	1 month	1	0.32	Recovered no further joint engaged after 2 years

microscopic diagnosis was in 5 out of 6 cases examined nonspecific synovitis. In case 8 a picture compatible with rheumatoid synovitis was found.

Culture of the synovial fluids revealed no bacterial or viral growth at time of enzyme examination. The white cell counts of the synovial fluids were variable and apparently without any correlation with the acid phosphatase activity.

The acid phosphatase activity was high in the poly and monarticular cases with the initial diagnosis JRA (cases 1-7 Table 1).

The acid phosphatase activity was also high in 3 cases with monarticular joint effusions of initially unknown etiology (nos 8-10). Two of these cases (nos 8 and 10) showed persisting signs of synovitis 1 year and 5 months respectively after joint fluid examination. Synovectomy was performed in both cases. Case 8 showed a histopathological picture compatible

with rheumatoid synovitis. Patient 10 showed a picture of chronic non specific synovitis but was classified as a case of probable JRA due to the persistence of synovitis over a period longer than 4 months. The third case (no 9) showed on X ray pictures 2 months after the joint fluid examination a bone destruction in the femoral epiphysis. The suspected diagnosis of bacterial osteoarthritis could not be confirmed by bacterial culture. After 9 months signs of synovitis still persisted, but the bone destruction showed definite regression.

Two patients showed low initial values of acid phosphatase activity (nos 11 and 12). Case 11 had a coxitis which by its clinical development and rapid regress of symptoms was considered to be a transient arthritis. Case 12 had a bacterial arthritis of 1 month's duration. Both patients recovered completely and showed after 2 and 1 year's (respectively) follow up no further joint engagement.

From 3 patients with the diagnosis JRA synovial fluid was examined after synovectomy (nos 2, 4 and 6). In these cases the acid phosphatase activity decreased after operation. In 2 cases (with more than one joint engaged) the decrease of enzyme activity was moderate (no 2 and 4). One patient with monoarticular engagement (no 6) showed low values comparable with those found in non-inflammatory joint effusions. No further joint engagement was observed in this patient.

### DISCUSSION

The mean acid phosphatase level in 7 children with the initial diagnosis JRA (Table 2) has been compared with those found in an earlier investigation of adult patients with rheumatoid arthritis and with non-inflammatory joint effusions (1). In JRA the acid phosphatase activity was high with a level very similar to that found in classic and definite rheumatoid arthritis of adults. The level is significantly ( $p < 0.001$ ) higher than that found in non-inflammatory joint effusions of adults.

Two non-rheumatoid patients (nos 11 and 12) had low enzyme levels comparable with those of adults with non-inflammatory joint effusions.

A high acid phosphatase activity was found also in 3 patients with an initial clinical picture of monoarticular synovitis. In one of these cases the diagnosis JRA was confirmed 1 year later by synovial biopsy. In another case the course of the disease points towards rheumatoid arthritis. In the third case the possibility of bacterial osteo-arthritis could not be excluded with sufficient certainty but the course of the disease was indicative of JRA.

Elevated levels of acid phosphatase activity in the synovial fluid were found in JRA. High levels were also found in monoarticular chronic synovitis which later proved to be JRA. Determinations of acid phosphatase activity in the synovial fluid may therefore contribute to the ascertainment of the diagnosis of JRA. Thus it seems worthwhile to examine the acid phosphatase level of the synovial fluid in a larger

Table 2. Acid phosphatase activity in the synovial fluid of adult patients with classic and definite rheumatoid arthritis, juvenile rheumatoid arthritis and adults with non-inflammatory joint effusions. Activity expressed in micromoles of a naphthyl phosphate hydrolyzed per ml fluid per hour

Patient group	Acid phosphatase activity		No. examined
	$M \pm s.e.$	S.D.	
Classic and definite rheumatoid arthritis adults <sup>a</sup>	$2.86 \pm 0.20$	1.68	101
Juvenile rheumatoid arthritis	$2.84 \pm 0.50$	1.32	7
Non-inflammatory joint effusions adults <sup>b</sup>	$0.22 \pm 0.05$	0.09	17

Data from Beckman *et al.* (1)

number of children in order to assess its value in the early diagnosis of JRA.

### SUMMARY

The acid phosphatase activity in the synovial fluid of nine children with juvenile rheumatoid arthritis was high and of the same order of magnitude as that found in adult patients with classic and definite rheumatoid arthritis. The present results suggest that determinations of acid phosphatase activity may be a useful contributory test in the diagnosis of juvenile rheumatoid arthritis.

### REFERENCES

- Beckman O, Beckman, L. & Lampert, R. Acid phosphatase activity in the synovial fluid of patients with different joint disorders. To be published.
- Canady J T, Brody G L. & Mariel, U. Monoarticular juvenile rheumatoid arthritis. *J Pediatr* 70: 867 1967.
- Edgren G. Rheumatoid arthritis and Still's disease in children. *Arthritis Rheum* 1: 497 1958.
- Laaksonen A. A prognostic study of juvenile rheumatoid arthritis. *Acta Paediat Scand* 55 Suppl 166 1966.

Submitted Sept 13 1969

Accepted Oct 1 1969

(R. L.) Dept of Orthopedic Surgery

University Hospital

Umeå 6

Sweden

Key Words: Acid phosphatase, juvenile rheumatoid arthritis, synovial fluid.

Table 1 Clinical data and acid phosphatase activity

Activity expressed in micromoles of  $\alpha$  naphthyl phosphate hydrolyzed per ml fluid per hour. The clinical diagnosis and duration of illness refers to the time of joint fluid analysis

Case no	Age (years)	Clinical diagnosis	Duration of illness	No of joints involved	Acid phosphatase	Remarks
1	8	JRA	7 years	2	1.13	
2	8	JRA	7 years	3	4.12	
Same patient 1 month after synovectomy					1.39	
3	8	JRA	3 years	2	2.52	
4	10	JRA	8 years	2	3.09	
Same patient 11 months after synovectomy					2.12	
5	13	JRA	2 years	3	1.06	
6	13	JRA	1 year	1	3.97	No further joint engaged after 2 years
Same patient 9 months after synovectomy					0.12	
7	14	JRA	9 months	1	3.97	One year later still joint effusion no further joint engaged
8	1	Monarticular synovitis	1 month	1	1.69	Op one year later Diagn JRA confirmed by synovial biopsy
9	4	Monarticular synovitis (bacterial osteo-arthrit <sup>is</sup> )	6 weeks	1	3.93	After 9 months still signs of synovitis. No other joint engaged. Diagnosis still uncertain
10	12	Monarticular synovitis	5 weeks	1	1.47	Op 5 months later no further joint engaged after 9 months
11	4	Transient arthritis of the hip	1 day	1	0.30	Recovered no further joint engaged after 1 year
12	7	Bacterial arthritis	1 month	1	0.32	Recovered no further joint engaged after 2 years

microscopic diagnosis was in 5 out of 6 cases examined nonspecific synovitis. In case 8 a picture compatible with rheumatoid synovitis was found.

Culture of the synovial fluids revealed no bacterial or viral growth at time of enzyme examination. The white cell counts of the synovial fluids were variable and apparently without any correlation with the acid phosphatase activity.

The acid phosphatase activity was high in the poly and monarticular cases with the initial diagnosis JRA (cases 1-7 Table 1).

The acid phosphatase activity was also high in 3 cases with monarticular joint effusions of initially unknown etiology (nos 8-10). Two of these cases (nos 8 and 10) showed persisting signs of synovitis 1 year and 5 months respectively after joint fluid examination. Synovectomy was performed in both cases. Case 8 showed a histopathological picture compatible

with rheumatoid synovitis. Patient 10 showed a picture of chronic non specific synovitis but was classified as a case of probable JRA due to the persistence of synovitis over a period longer than 4 months. The third case (no 9) showed on X ray pictures 2 months after the joint fluid examination a bone destruction in the femoral epiphysis. The suspected diagnosis of bacterial osteoarthrit<sup>is</sup> could not be confirmed by bacterial culture. After 9 months signs of synovitis still persisted but the bone destruction showed definite regression.

Two patients showed low initial values of acid phosphatase activity (nos 11 and 12). Case 11 had a covitis which by its clinical development and rapid regress of symptoms was considered to be a transient arthritis. Case 12 had a bacterial arthritis of 1 month's duration. Both patients recovered completely and showed after 2 and 1 year's (respectively) follow up no further joint engagement.

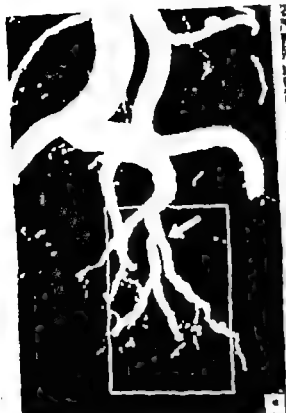


Fig. 1. Bronchial artery supplied area of the lung with prominent arterioles and hyaline membranes. Age 4 days, birth weight 450 g (A 161 V2) (a) Microangiogram.  $\times 30$ . The bronchial arteries in the upper



part of the field and ramifications to the pulmonary parenchyma. (Corresponding arrows in A and B) (b) Serial histological section from framed area in A. Elastin van Gieson.  $\times 76$ .

The present work on the bronchial artery supply of the pulmonary parenchyma complements previous post mortem microangiographical studies on the pulmonary vasculature in IRDS. With a concentrated barium suspension Lawler *et al.* (4) obtained poor filling of the peripheral pulmonary arteries and arterioles and interpreted the results as indicating pulmonary hypoperfusion. On the other hand Linderen (6) and Iversen & Wallgren (3) who used the same technique in principle as here demonstrated a normal pulmonary artery pattern in IRDS lungs. Nor are there signs of an abnormal pulmonary vein pattern in IRDS lungs (3, 5). The bulk of the evidence then is compatible with a normal vascular pattern in IRDS lungs.

Borcs (?) hypothesis in his report of the

Lausanne baby that systemic artery supply of the pulmonary parenchyma prevents the development of PHM has been questioned on other grounds by Adams & El Salawy (1). They elicited evidence that the lung lobe in question was totally sequestered, i.e. had neither respiratory nor arterial connections with the rest of the lung.

Since PHM equally affects areas of the human neonatal lung supplied by systemic and pulmonary arteries there are no grounds for supposing that abnormalities in the pulmonary artery system alone constitute a primary factor in the still obscure pathogenesis of IRDS.

#### SUMMARY

A combined microangiographical and histological study of the bronchial arterial system

## SHORT COMMUNICATION

# HYALINE MEMBRANES IN BRONCHIAL ARTERY SUPPLIED AREAS OF THE HUMAN NEONATAL LUNG

## *A Microangiographical and Histological Study*

C A GRANT and B ROBERTSON

*From the Department of Paediatric Pathology, Karolinska sjukhuset, Stockholm, Sweden*

Asymmetrical distribution of atelectasis and pulmonary hyaline membranes (PHM) in the idiopathic respiratory distress syndrome (IRDS) has been noticed on a few occasions (2-7, 10). In some of these cases the asymmetry of the lesions was attributed to impairment of pulmonary lymphatic flow (7) or abnormal systemic artery supply of the unaffected lung lobe (2). However, in the newborn infant small areas of the pulmonary parenchyma are normally supplied by bronchial artery branches, so called bronchopulmonary arteries (9, 11). In view of the possible influence of vascular aberrations on the development of PHM, we have carried out the present study on the occurrence of PHM in areas of the neonatal lung supplied by bronchopulmonary arteries.

## MATERIAL AND METHODS

The bronchial arterial system in 10 newborn infants with IRDS and PHM was injected via the thoracic aorta with 7.5 per cent aqueous suspension of barium sulphate (Micropaque 2). Bronchial artery supplied areas of the pulmonary parenchyma were identified in microangiograms representing 1500  $\mu$  thick frontal slices of the lung (for details of the microangiographic technique see 8). Serial histological sections from such areas to confirm the existence of bronchopulmonary arteries were then examined for the presence of PHM and compared with sections from other parts of the lung. The birth weight of the subjects ranged from 1100 g to 2780 g and their age from 8 hours to 5 days.

## RESULTS

In 8 of the infants small areas of pulmonary parenchyma supplied by branches of the bronchial artery could be identified. The pattern fitted in with what is normally encountered in the lung of the newborn infant, i.e. scattered small wedges of pulmonary parenchyma usually adjacent to bronchial bifurcations are marginally supplied with filling of the peripheral alveolar capillaries. In two infants the bronchial artery branches extended deeper into the wedges to supply the terminal respiratory passages as well. In places there was transmission of contrast to peripheral pulmonary-artery branches through a common capillary bed. Taken as a whole, branches of the bronchial arteries supplied only a very minor portion of the pulmonary parenchyma. Regardless of the origin of the arterial supply, however, the distribution of PHM and atelectasis was uniform throughout the parenchyma.

There was no evidence for abnormal arterial bronchopulmonary anastomoses or arterio-venous shunts.

## COMMENT

The normal mural structure and distribution pattern of bronchopulmonary arteries in the lungs of these infants with IRDS and PHM followed the normal pattern (8).

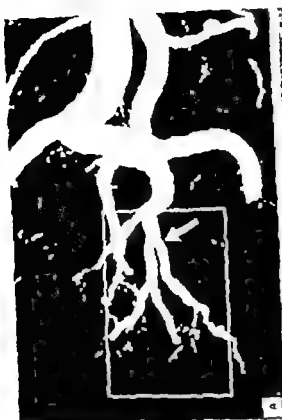


Fig 1 Bronchial artery supplied area of the lung with prominent arterioles and hyaline membranes. Age 4 days birth weight 450 g (A 161 V2) (a) Macroangiogram 30 The bronchial arteries in the upper

part of the field and ramifications to the pulmonary parenchyma (Corresponding arrows in A and B) (b) Serial histological section from framed area in A Elston van Gieson 76



The present work on the bronchial artery supply of the pulmonary parenchyma complements previous post mortem macroangiographical studies on the pulmonary vasculature in IRDS. With a concentrated barium suspension Lawryns (4) obtained poor filling of the peripheral pulmonary arteries and arterioles and interpreted the results as indicating pulmonary hypoperfusion. On the other hand Larderen (6) and Ismark & Wallgren (3) who used the same technique in principle as here demonstrated a normal pulmonary artery pattern in IRDS lungs. Nor are there signs of an abnormal pulmonary vein pattern in IRDS lungs (3, 5). The bulk of the evidence then is compatible with a normal vascular pattern in IRDS lungs.

Borer's (2) hypothesis in his report of the

Lausanne baby that systemic artery supply of the pulmonary parenchyma prevents the development of PHM has been questioned on other grounds by Adams & El Salawy (1). They elicited evidence that the lung lobe in question was totally sequestered i.e. had neither respiratory nor arterial connection with the rest of the lung.

Since PHM equally affects areas of the human neonatal lung supplied by systemic and pulmonary arteries there are no grounds for supposing that abnormalities in the pulmonary artery system alone constitute a primary factor in the still obscure pathogenesis of IRDS.

# SUMMARY

A combined macroangiographical and histological study of the bronchial arterial system



was carried out on 10 neonatal subjects dying from the idiopathic respiratory distress syndrome with pulmonary hyaline membranes. The bronchial artery pattern was normal in all cases. Small areas of the pulmonary parenchyma supplied by bronchial artery branches, so-called bronchopulmonary arteries, were identified in 8 infants. The pulmonary lesions were the same in these areas as in the rest of the lung, indicating that systemic artery supply of the pulmonary parenchyma does not prevent the formation of pulmonary hyaline membranes.

## REFERENCES

- 1 Adams F H & El Sahwy A. Pulmonary hyaline membranes and vascular anomalies of the lung. The Lausanne Baby. *Pediatrics* 39 791 1967
- 2 Bozic C. Pulmonary hyaline membranes and vascular anomalies of the lung. Description of a case. *Pediatrics* 37 1094-1097 1963
- 3 Ivemark B I & Wallgren G. The pulmonary vascular pattern in idiopathic respiratory distress. A microangiographic study. *Acta Path Microbiol Scand* 76 203-214 1969
- 4 Lauweryns J M. Pulmonary arterial vasculature in neonatal hyaline membrane disease. *Science* 153 1275-1277 1966
- 5 Lauweryns J M. Pulmonary venous vasculature in neonatal hyaline membrane disease. *Science* 160 190-192 1968

- 6 Lindgren I. The pulmonary arterial and capillary pattern in hyaline membrane disease. *Acta Paediatr Scand* 54 526-530 1965
- 7 Molz G. Einseitiges Vorkommen alveolärer hyaliner Membranen bei einem unter der Geburt verstorbenen Neugeborenen. *Helv Paediatr Acta* 22 441-446 1967
- 8 Robertson B. The intrapulmonary arterial pattern in normal infancy and in transposition of the great arteries. *Acta Paediatr Scand Suppl* 184 1968
- 9 Robertson B. The incidence and structure of bronchopulmonary arteries in infancy and early childhood. *Biol Neonat* 14 62-68 1969
- 10 Rudhe U, Margolin F R & Robertson B. Atypical roentgen appearance of the lungs in hyaline membrane disease of the newborn. To be published in *Acta Radiol*
- 11 Wagenvoort C A & Wagenvoort N. Arterial anastomoses bronchopulmonary arteries and pulmobronchial arteries in perinatal lungs. *Lab Invest* 16 13-24 1967

Submitted Oct 28 1969

Accepted Nov 4 1969

Dept of Paediatric Pathology  
Karolinska sjukhuset  
S 104 01 Stockholm 60  
Sweden

Key words: Newborn infant idiopathic respiratory distress syndrome; pulmonary hyaline membranes; bronchial arterial system; bronchopulmonary arteries; microangiography.

## CASE REPORT

RECURRENT BACTEREMIA FOLLOWING AMPICILLIN  
TREATMENT OF HEMOPHILUS INFLUENZAE MENINGITIS

ROBERT A. ACHEL

*From the Department of Pediatrics Yale University School of Medicine and  
the Yale New Haven Hospital New Haven Connecticut U.S.A.*

Ampicillin has been accepted as an effective therapeutic agent in the treatment of *Hemophilus influenzae* infections (1-7, 11). *In vitro* sensitivity remains virtually complete (9, 11). However eight cases of ampicillin failure in *Hemophilus influenzae* meningitis have been reported (8, 10). Failure has been ascribed to inadequate cerebrospinal fluid levels of ampicillin secondary to inadequate dosage or improper route of administration in seven of these cases (2-8). In the eighth case Young *et al.* (10) reported the occurrence of orbital cellulitis and bacteremia three days after a 14 day course of ampicillin suggesting inadequate antibiotic penetration in an area where organisms had been sequestered. Subdural punctures were performed in three of the eight reported cases, only one being productive of fluid which proved sterile.

The following is a case report of an infant with *Hemophilus influenzae* meningitis where ampicillin failed to eradicate the infection possibly because of the presence of left subdural effusion.

## CASE REPORT

B. A., a seven month-old white male presented with a 3-day history of a bulging anterior fontanelle, irritability, anorexia and fever of 40.2°C. A lumbar puncture showed grossly purulent cerebrospinal fluid (Table 1) cultures of which subsequently grew *H. influenzae* type B. Growth inhibition was

greater than 15 mm with a 10 µg ampicillin disc. Intravenous ampicillin therapy was begun with an initial dose of 1 g (103 mg per kg) and continued at 2 (206 mg per kg) per day administered over a ten minute period in divided doses every four hours for a period of ten days. Ampicillin was administered intramuscularly on the 11th and 12th hospital days because of the paucity of suitable veins. A second lumbar puncture was performed 48 hours after ampicillin therapy (Table 1) and cultures were sterile.

A right hemiparesis became evident by the 3rd hospital day. Transillumination was moderately increased over the left frontoparietal region. A left subdural puncture through the coronal suture yielded 1 ml of sterile fluid (Table 2). Fever declined gradually to normothermic levels by the 5th day but rose to 39°C on the 9th day. Transillumination was no longer increased and the right hemiparesis had disappeared. A persistent nasal discharge had developed but nasopharyngeal cultures revealed normal flora. A 3rd lumbar puncture performed on the 10th day (Table 1) was sterile. Tube dilutions of cerebrospinal fluid demonstrated growth inhibition of the original organisms at 1:4. The patient was afebrile by the 12th day, cultures remained sterile and ampicillin was discontinued. The patient's temperature rose to 38°C on the 13th day, a fourth lumbar puncture was performed and chloramphenicol in doses of 100 mg/kg a day was given intravenously in divided doses administered every four hours. Fever abated within 72 hours, cerebrospinal and blood cultures remained sterile and a repeat lumbar puncture was (Table 1) within normal limits. Chloramphenicol was therefore discontinued.

A low grade fever (between 38 and 38.5°C) developed within 4 hours and persisted for the next 7 days in the absence of clinical symptoms. Nasopharyngeal blood and urine cultures were unproductive. X-rays of the chest, sinuses, and mastoids and an electroencephalogram were within normal limits. The patient's temperature was 39.5°C on the 24th day.

was carried out on 10 neonatal subjects dying from the idiopathic respiratory distress syndrome with pulmonary hyaline membranes. The bronchial artery pattern was normal in all cases. Small areas of the pulmonary parenchyma supplied by bronchial artery branches so called 'bronchopulmonary arteries' were identified in 8 infants. The pulmonary lesions were the same in these areas as in the rest of the lung indicating that systemic artery supply of the pulmonary parenchyma does not prevent the formation of pulmonary hyaline membranes.

### REFERENCES

- 1 Adams F H & El-Silawy A. Pulmonary hyaline membranes and vascular anomalies of the lung. The Lausanne Baby. *Pediatrics* 39 791 1967
- 2 Bozic C. Pulmonary hyaline membranes and vascular anomalies of the lung. Description of a case. *Pediatrics* 32 1094 1097 1963
- 3 Iversen H I & Wallgren G. The pulmonary vascular pattern in idiopathic respiratory distress. A microangiographic study. *Acta Path Microbiol Scand* 76 203-214 1969
- 4 Lauweryns J M. Pulmonary arterial vasculature in neonatal hyaline membrane disease. *Science* 153 1275-1277 1966
- 5 Lauweryns J M. Pulmonary venous vasculature in neonatal hyaline membrane disease. *Science* 160 190-192 1969

- 6 Lindgren I. The pulmonary arterial and capillary pattern in hyaline membrane disease. *Acta Paediat Scand* 54 526-530 1965
- 7 Molz G. Einseitiges Vorkommen alveolarer hyaliner Membranen bei einem unter der Geburt verstorbenen Neugeborenen. *Helv Paediat Acta* 22 441-446 1967
- 8 Robertson B. The intrapulmonary arterial pattern in normal infancy and in transposition of the great arteries. *Acta Paediat Scand Suppl* 184 1968
- 9 Robertson B. The incidence and structure of bronchopulmonary arteries in infancy and early childhood. *Biol Neonat* 14 62-68 1969
- 10 Rudhe U, Murgolin F R & Robertson B. Atypical roentgen appearance of the lungs in hyaline membrane disease of the newborn. To be published in *Acta Radiol*
- 11 Wagenvoort C A & Wagenvoort N. Arterial anastomoses: bronchopulmonary arteries and pulmobronchial arteries in perinatal lungs. *Lab Invest* 16 13-24 1967

Submitted Oct 28 1969

Accepted Nov 4 1969

Dept of Paediatric Pathology  
Karolinska sjukhuset  
S 104 01 Stockholm 60  
Sweden

**Key words:** Newborn infant idiopathic respiratory distress syndrome pulmonary hyaline membranes bronchial arterial system bronchopulmonary arteries microangiography

## CASE REPORT

### RECURRENT BACTEREMIA FOLLOWING AMPICILLIN TREATMENT OF HEMOPHILUS INFLUENZAE MENINGITIS

ROBERT A. ACHTEL

From the Department of Pediatrics Yale University School of Medicine and  
the Yale New Haven Hospital New Haven Connecticut U.S.A.

Ampicillin has been accepted as an effective therapeutic agent in the treatment of *Hemophilus influenzae* infections (1-7, 11). *In vitro* sensitivity remains virtually complete (9, 11). However, eight cases of ampicillin failure in *Hemophilus influenzae meningitis* have been reported (8, 10). Failure has been ascribed to inadequate cerebrospinal fluid levels of ampicillin secondary to inadequate dosage or an improper route of administration in seven of the 8 cases (2-8). In the eighth case Young *et al.* (10) reported the occurrence of orbital cellulitis and bacteremia three days after a 14 day course of ampicillin suggesting inadequate antibiotic penetration in an area where organisms had been sequestered. Subdural punctures were performed in three of the eight reported cases only one being productive of fluid which proved sterile.

The following is a case report of an infant with *Hemophilus influenzae meningitis* where ampicillin failed to eradicate the infection possibly because of the presence of left subdural effusion.

#### CASE REPORT

A 24-month-old white male presented with a generalized seizure, bulging anterior fontanelle, bilateral otitis media and fever of 40.2°C. A lumbar puncture showed grossly purulent cerebrospinal fluid (Table 1) cultures of which subsequently grew *H. influenzae* type B. Growth inhibition was

greater than 15 mm with a 10 µg ampicillin disc. Intravenous ampicillin therapy was begun with an initial dose of 1 g (103 mg per kg) and continued at 2 (206 mg per kg) per day administered over a ten minute period in divided doses every four hours for a period of ten days. Ampicillin was administered intramuscularly on the 11th and 12th hospital days because of the paucity of suitable veins. A second lumbar puncture was performed 48 hours after ampicillin therapy (Table 1) and cultures were sterile.

A right hemiparesis became evident by the 3rd hospital day. Transillumination was moderately increased over the left frontoparietal region. A left subdural puncture through the coronal suture yielded 1 ml of sterile fluid (Table 2). Fever declined gradually to normothermic levels by the 5th day but rose to 39°C on the 9th day. Transillumination was no longer increased and the right hemiparesis had disappeared. A purulent nasal discharge had developed but nasopharyngeal cultures revealed normal flora. A 3rd lumbar puncture performed on the 10th day (Table 1) was sterile. Tube dilutions of cerebrospinal fluid demonstrated growth inhibition of the original organisms at 1:4. The patient was afebrile by the 12th day, cultures remained sterile and ampicillin was discontinued. The patient's temperature rose to 38°C on the 13th day. A fourth lumbar puncture was performed and chloramphenicol in dosage of 100 mg/kg a day was given intravenously in divided doses administered every four hours. Fever abated within 7 hours, cerebrospinal and blood cultures remained sterile and a repeat lumbar puncture was (Table 1) within normal limits. Chloramphenicol was therefore discontinued.

A low grade fever (between 38° and 38.5°C) developed within 24 hours and persisted for the next 7 days in the absence of clinical symptoms. Nasopharyngeal, blood and urine cultures were unproductive. X-rays of the chest, sinuses and mastoids and immunoelectrophoresis were within normal limits. The patient's temperature was 39.5°C on the 24th day.

was carried out on 10 neonatal subjects dying from the idiopathic respiratory distress syndrome with pulmonary hyaline membranes. The bronchial artery pattern was normal in all cases. Small areas of the pulmonary parenchyma supplied by bronchial artery branches so-called bronchopulmonary arteries were identified in 8 infants. The pulmonary lesions were the same in these areas as in the rest of the lung indicating that systemic-artery supply of the pulmonary parenchyma does not prevent the formation of pulmonary hyaline membranes.

### REFERENCES

- 1 Adams F H & El-Silawy A. Pulmonary hyaline membranes and vascular anomalies of the lung. The Lausanne Baby. *Pediatrics* 39 791 1967
- 2 Bozic C. Pulmonary hyaline membranes and vascular anomalies of the lung. Description of a case. *Pediatrics* 37 1094-1097 1963
- 3 Isomärk H I & Wallgren G. The pulmonary vascular pattern in idiopathic respiratory distress. A microradiographic study. *Acta Path Microbiol Scand* 76 203-214 1969
- 4 Lauweryns J M. Pulmonary arterial vasculature in neonatal hyaline membrane disease. *Science* 153 1275-1277 1966
- 5 Lauweryns J M. Pulmonary venous vasculature in neonatal hyaline membrane disease. *Science* 160 190-192 1968

- 6 Lindgren I. The pulmonary arterial and capillary pattern in hyaline membrane disease. *Acta Paediatr Scand* 54 526-530 1965
- 7 Molz G. Einseitiges Vorkommen alveolärer hyaliner Membranen bei einem unter der Geburt verstorbenen Neugeborenen. *Helv Paediatr Acta* 22 441-446 1967
- 8 Robertson B. The intrapulmonary arterial pattern in normal infancy and in transposition of the great arteries. *Acta Paediatr Scand* Suppl 154 1968
- 9 Robertson B. The incidence and structure of bronchopulmonary arteries in infancy and early childhood. *Biol Neonat* 14 62-68 1969
- 10 Rudhe U, Margolin F R & Robertson B. Atypical roentgen appearance of the lungs in hyaline membrane disease of the newborn. To be published in *Acta Radiol*
- 11 Wagenvoort C A & Wagenvoort N. Arterial anastomoses: bronchopulmonary arteries and pulmobronchial arteries in perinatal lungs. *Lab Invest* 16 13-24 1967

Submitted Oct 28 1969  
Accepted Nov 4 1969

Dept of Paediatric Pathology  
Karolinska sjukhuset  
S 104 01 Stockholm 60  
Sweden

**Key words:** Newborn infant, idiopathic respiratory distress syndrome, pulmonary hyaline membranes, bronchial arterial system, bronchopulmonary arteries, microradiography.

strongly suggestive of a subdural effusion. Negative findings from the first subdural puncture did not rule out a subdural effusion as the needle may not have entered the fluid lake or the viscosity of the fluid may have precluded withdrawal. A positive culture from the thick material obtained after left subdural puncture on the 24th day proves infection within this space. These findings would support the hypothesis of sequestration of organisms in the subdural space with subsequent reseeding of blood stream. The negative subdural puncture on the 37th day suggests that sterilization of this space may have occurred following combined therapy with both ampicillin and chloramphenicol or evacuation of the space.

Failure of ampicillin in *Hemophilus influenzae* meningitis may be the result of inadequate perfusion of the cerebrospinal fluid by the antibiotic as a result of insufficient dosage or administration by other than the intravenous route. Less frequently where adequate cerebrospinal fluid levels of an effective antibiotic are maintained relapse may be attributed to heterogenous or cerebrospinal fluid reseeding by organisms sequestered in areas which were inadequately perfused. A thorough search for foci of persistent infection must include subdural puncture whenever ampicillin failure is suspected in *hemophilus meningitis*.

# REFERENCES

1. Barret F, Eardley W, Yow M. & Leverett H. Ampicillin in the treatment of acute suppurative meningitis. *J Pediatr* 69: 343 1966.
- Cherry J H & Sherman C P. Bacteriologic

- Relapse in *Hemophilus influenzae* meningitis. *New Eng J Med* 278: 1001 1968.
3. Coleman S J, Auld P. B. Conner J H, Rosenman S H & Warren C H. Relapse of *Hemophilus influenzae* type B meningitis during intravenous therapy with ampicillin. *J Pediatr* 74: 781 1969.
4. Gold A J, Lieberman E. & Wright H T. Bacteriologic relapse during ampicillin treatment of *Hemophilus influenzae* meningitis. *J Pediatr* 74: 779 1969.
5. Greene H L. Failure of ampicillin in meningitis. *Lancet* i 861 1968.
6. Hall B D. Failure of ampicillin in meningitis. *Lancet* i 1032 1968.
7. Mathews A W Jr, Leedom J M, Thrapp L D, Ivler D, Portnoy B. & Wehrle P F. Experience with ampicillin in bacterial meningitis. *Antimicrob Agents Chemother* 1965: 5: 610 1966.
8. Sanders D Y & Garbee H W. Failure of response to ampicillin in *Hemophilus influenzae* meningitis. *Am J Dis Child* 117: 331 1969.
9. Wehrle P F, Mathews A W, Jr & Leedom J M. Management of bacterial meningitis in *Clinical Neurosurgery* vol 14. *The Congress of Neurological Surgeons* 1967: p 72.
10. Young L M, Haddow J E. & Klein J O. Relapse following ampicillin treatment of acute *Hemophilus influenzae* meningitis. *Pediatrics* 41: 516 1968.
11. Yow M, Mertha D. Ampicillin in the treatment of meningitis due to *Hemophilus influenzae*. An appraisal after 6 years of experience. *J Pediatr* 74: 848 1969.

Submitted Sept 11 1969

Accepted Oct 9 1969

Dept of Pediatrics  
Yale University School of Medicine  
333 Cedar Street  
New Haven  
Connecticut 06510  
U S A

*Key words:* *Hemophilus influenzae* meningitis; ampicillin; recurrence of infection.

Table 1 Lumbar punctures

LP	Hosp day	TNC	Cells		RBCs	Protein	CSF sugar	Blood sugar	Culture
			Gran	Lymph					
1	1	1660	1644	16	0	45	40	109	H ufl
2	2	170	136	34	5	48	48	62	Neg
3	10	9	1	8	0	33	46	87	Neg
4	13	310	31	279	90	32	38	87	Neg
5	16	0	0	0	39	26	63	90	Neg
6	24	16	0	16	5	40	50	110	Neg
7	36	55	5	50	0	81	53	85	Neg

TNC = Total nucleated cells Gran = granulocytes Lymph = lymphocytes

after admission or 8 days off therapy and a 6th lumbar puncture was performed (Table 1). The following day bilateral subdural punctures (Table 2) yielded 1 ml of thick fluid from each side. No pharyngeal blood and fluid from the left subdural tap cultured *Hemophilus influenzae* type B. Cultures of cerebrospinal fluid and bilateral middle ear aspirates remained sterile. Chloramphenicol was again begun according to the regimen cited previously. The patient's sensorium diminished fever persisted above 39°C and by the 28th day he was unresponsive. The gravity of the situation was considered and ampicillin added to the therapeutic regimen according to the method described previously. On this combined therapy the patient gradually improved and became responsive and afebrile over the next 3 days. Chloramphenicol was continued until the 34th and ampicillin until the 37th hospital day. A 7th lumbar puncture (Table 1) performed on the 36th day was sterile. Bilateral subdural punctures on the 37th day yielded 3 ml of sterile fluid from the left side (Table 2) and none from the right.

The patient remained in the hospital for 6 days following the discontinuation of antibiotics. During this time he was afebrile and clinically well. Neither further diagnostic evaluation nor treatment was thought indicated and he was discharged home on the 43rd day. Over the 5 months since discharge he has remained without clinical evidence of infection.

### DISCUSSION

Various reasons for ampicillin failure in the treatment of *Hemophilus influenzae* meningitis

have been suggested. Cherry *et al* (2) suggested the inadequacy of the intramuscular and oral routes in achieving bactericidal concentrations of ampicillin in the cerebrospinal fluid. Gold *et al* (4) experienced relapse when using a dosage of 150 mg of ampicillin per kg per day. Greater dosage for certain patients was suggested. Coleman *et al* (3) have stressed that the fluctuations of cerebrospinal fluid levels of ampicillin vary directly with the cellular response and indirectly with time.

The patient presented in this report was treated intravenously with 309 mg of ampicillin per kg on the first day and 206 mg per kg per day for the succeeding 9 days and then intramuscularly on the 11th and 12 days. Tube dilutions of cerebrospinal fluid on the 10th day of treatment demonstrated inhibition of the original organisms at 1:4. These findings suggest adequate antibiotic perfusion of the cerebrospinal fluid. Young *et al* (10) suggested that recurrent infection was due to residual focal sequestration of organisms with subsequent reseeding of the blood stream. In our patient transient increase of left frontoparietal transillumination and right hemiparesis were

Table 2 Subdural punctures

	Hospital day	Side	Vol (ml)	TNC	Gran	Lymph	RBCs	Protein (mg/100 ml)	Culture
1	3	Left	1	98	45	48	800	250	Sterile
2	25	Left	1	—	—	—	—	—	H ufl type B
2	25	Right	1	—	—	—	—	—	Sterile
3	37	Left	3	3	0	3	0	570	Sterile
3	37	Right	0	—	—	—	—	—	—

strongly suggestive of a subdural effusion. Negative findings from the first subdural puncture did not rule out a subdural effusion as the needle may not have entered the fluid lake or the viscosity of the fluid may have precluded withdrawal. A positive culture from the thick material obtained after left subdural puncture on the 24th day proves infection within this space. These findings would support the hypothesis of sequestration of organisms in the subdural space with subsequent reseeding of blood stream. The negative subdural puncture on the 37th day suggests that sterilization of this space may have occurred following combined therapy with both ampicillin and chloramphenicol or evacuation of the space.

Failure of ampicillin in *Hemophilus influenzae* meningitis may be the result of inadequate perfusion of the cerebrospinal fluid by the antibiotic as a result of insufficient dosage or administration by other than the intravenous route. Less frequently where adequate cerebrospinal fluid levels of an effective antibiotic are maintained relapse may be attributed to hematogenous or cerebrospinal fluid reseeding by organisms sequestered in areas which were inadequately perfused. A thorough search for foci of persistent infection must include subdural puncture whenever ampicillin failure is suspected in *hemophilus meningitis*.

# REFERENCES

1. Barrett F, Eardley W, Yow M & Leverett H. Ampicillin in the treatment of acute suppurative meningitis. *J Pediatr* 69: 343 1966.
2. Cherry J D & Shewmon C H. Bacteriologic

- Relapse in *Hemophilus influenzae* meningitis. *New Eng J Med* 278: 1001 1968.
3. Coleman S J, Auld E, B. Conner J D, Rosenman S H & Warren O H. Relapse of *Hemophilus influenzae* type b meningitis during intravenous therapy with ampicillin. *J Pediatr* 74: 781 1969.
4. Gold A J, Lieberman, E. & Wright H T. Bacteriologic relapse during ampicillin treatment of *Hemophilus influenzae* meningitis. *J Pediatr* 74: 779 1969.
5. Greene H L. Failure of ampicillin in meningitis. *Lancet* 1: 861 1968.
6. Hall B D. Failure of ampicillin in meningitis. *Lancet* 1: 1033 1968.
7. Mathes A W Jr, Leedom J M, Thrupp L, H. Ivler D, Portnoy B & Wehrle P F. Experience with ampicillin in bacterial meningitis. *Antimicrob Agents Chemother* 1965: 5: 610 1966.
8. Sanders D Y & Garber H W. Failure of response to ampicillin in *Hemophilus influenzae* meningitis. *Am J Dis Child* 117: 331 1969.
9. Wehrle P F, Mathes A. W Jr & Leedom J M. Management of bacterial meningitis in *Clinical Neurosurgery* vol 14. *The Congress of Neurological Surgeons* 1967: p 72.
10. Young L M, Haddow J E. & Niem J O. Relapse following ampicillin treatment of acute *Hemophilus influenzae* meningitis. *Pediatrics* 41: 516 1968.
11. Yow Martha D. Ampicillin in the treatment of meningitis due to *Hemophilus influenzae*. An appraisal after 6 years of experience. *J Pediatr* 74: 848 1969.

Submitted Sept. 11 1969

Accepted Oct. 9 1969

Dept. of Pediatrics  
Yale University School of Medicine  
333 Cedar Street  
New Haven  
Connecticut 06510  
U.S.A.

Key words: *Hemophilus influenzae* meningitis; ampicillin; relapse; recurrence of infection.



Table 1 Lumbar punctures

LP	Hosp day	TNC	Cells		RBCs	Protein	CSF sugar	Blood sugar	Culture
			Gran	Lymph					
1	1	1660	1644	16	0	45	40	109	<i>H. infl</i>
2	2	170	136	34	5	48	48	62	Neg
3	10	9	1	8	0	33	46	87	Neg
4	13	310	31	279	90	32	38	87	Neg
5	16	0	0	0	39	26	63	90	Neg
6	24	16	0	16	5	40	50	110	Neg
7	36	55	5	50	0	81	53	85	Neg

TNC = Total nucleated cells Gran = granulocytes Lymph = lymphocytes

after admission or 8 days off therapy and a 6th lumbar puncture was performed (Table 1). The following day bilateral subdural punctures (Table 2) yielded 1 ml of thick fluid from each side. No pharyngeal blood and fluid from the left subdural tap cultured *Hemophilus influenzae* type B. Cultures of cerebrospinal fluid and bilateral middle ear aspirates remained sterile. Chloramphenicol was again begun according to the regimen cited previously. The patient's sensorium diminished, fever persisted above 39°C and by the 29th day he was unresponsive. The gravity of the situation was considered and ampicillin added to the therapeutic regimen according to the method described previously. On this combined therapy the patient gradually improved and became responsive and afebrile over the next 5 days. Chloramphenicol was continued until the 34th and ampicillin until the 37th hospital day. A 7th lumbar puncture (Table 1) performed on the 36th day was sterile. Bilateral subdural punctures on the 37th day yielded 3 ml of sterile fluid from the left side (Table 2) and none from the right.

The patient remained in the hospital for 6 days following the discontinuation of antibiotics. During this time he was afebrile and clinically well. Neither further diagnostic evaluation nor treatment was thought indicated and he was discharged home on the 43rd day. Over the 5 months since discharge he has remained without clinical evidence of infection.

### DISCUSSION

Various reasons for ampicillin failure in the treatment of *Hemophilus influenzae* meningitis

have been suggested. Cherry *et al* (2) suggested the inadequacy of the intramuscular and oral routes in achieving bactericidal concentrations of ampicillin in the cerebrospinal fluid. Gold *et al* (4) experienced relapse when using a dosage of 150 mg of ampicillin per kg per day. Greater dosage for certain patients was suggested. Coleman *et al* (3) have stressed that the fluctuations of cerebrospinal fluid levels of ampicillin vary directly with the cellular response and indirectly with time.

The patient presented in this report was treated intravenously with 309 mg of ampicillin per kg on the first day and 206 mg per kg per day for the succeeding 9 days and then intramuscularly on the 11th and 12 days. Tube dilutions of cerebrospinal fluid on the 10th day of treatment demonstrated inhibition of the original organisms at 1:4. These findings suggest adequate antibiotic perfusion of the cerebrospinal fluid. Young *et al* (10) suggested that recurrent infection was due to residual focal sequestration of organisms with subsequent reseeding of the blood stream. In our patient transient increase of left frontoparietal transillumination and right hemiparesis were

Table 2 Subdural punctures

	Hospital day	Side	Vol (ml)	TNC	Gran	Lymph	RBCs	Protein (mg/100 ml)	Culture
1	3	Left	1	98	45	48	800	250	Sterile
2	25	Left	1	—	—	—	—	—	<i>H. infl</i> type B
2	25	Right	1	—	—	—	—	—	Sterile
3	37	Left	3	3	0	3	0	570	Sterile
3	37	Right	0	—	—	—	—	—	—

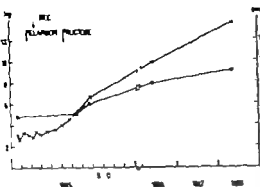


Fig 1 Growth of the patient x—x weight kg o—o height cm

total blood sugar (amihrose method ref 1) were noted in supposedly normal controls small but definite blood glucose increases were obtained on glucose tolerance tests. These findings were considered to be in accordance with a diagnosis of GGM or due to an unspecified damage of intestinal mucosa by the diarrhoea producing factor(s). Fructose tolerance test did not give rise to any definite increase of blood fructose or blood glucose. (Fructose increased to the difference between total hexose and glucose.) In all tolerance tests only small amounts of sugar were recovered in faeces. No non absorbable marker substances was used. The degree of recovery was therefore unknown making the interpretation of the sugar determinations in the faeces somewhat uncertain.

The findings in the fructose tolerance test and in the faeces in all the other tolerance tests were not in all respects as expected in GGM. In this condition a definite blood glucose response has earlier been found in fructose tolerance tests (5).

Xylose tolerance test was normal at this age (7.5%) of the dose (0.5 g/kg body weight) was excreted in the urine in five hours as well as at age

16 months (blood xylose 18 and 30 mg/100 ml after 30 and 60 min). At age 7 months pathologically low values were found (blood xylose 11 and 16 mg/100 ml at 30 and 60 min).

Glucose and fructose tolerance tests were repeated at age 7 months when the patient was in good health on her fructose formula. Now a definite blood glucose increase occurred when fructose was fed. The same was however true also with glucose. The diagnosis therefore although practically established by the effect of diet was not settled definitely by the blood sugar assays in the tolerance tests even at this time. Within two hours after the glucose feeding diarrhoea occurred which was of course the expected reaction. The same reaction had been noted at the earlier tolerance tests with glucose and galactose. In the glucose tolerance test 10 g of glucose and 2 g of polyethylene glycol were given. 13 g of glucose and 0.54 g of polyethylene glycol were recovered in the faeces during 24 hours. This indicated that 50% of the glucose dose was excreted in the faeces and glucose absorption was thus very low.

Although GGM was now considered the most likely diagnosis it was considered necessary to obtain better biochemical evidence. Intubation and perfusion of a segment of the intestine with sugars was therefore performed with a double lumen sond (G Messerle). The proximal lumen reached Treitz ligament and the distal one 25 cm further. Through the proximal outlet was pumped at 5 ml/min a solution of 2.2% glucose 2.2% fructose 0.09% NaCl 0.184% urea and 0.2% polyethylene glycol. Intestinal contents were sucked continuously through the distal hole. After 30 min of equilibration intestinal contents were collected for two periods of 20 min. It was found that glucose absorption was 1.9 and 6.7 mg/min/25 cm intestine respectively. The corresponding figures for fructose (measured according to Heyrovsky ref 1) were 25.7 and 30.5. The results clearly indicated that fructose was absorbed much better than glucose and definitely proved the diagnosis in our patient.



Fig 2 Appearance at age 4 months

## CASE REPORT

## DIAGNOSTIC PROBLEMS IN GLUCOSE GALACTOSE MALABSORPTION

*A Case Report*

A. KAUSER and P. A. ÖCKERMAN\*

*From the Department of Paediatrics (Head A. Kauser) Central County Hospital Eskilstuna Sweden*

Glucose-galactose malabsorption (GGM) is a rare hereditary disorder of the active absorption of certain sugars including galactose and glucose. GGM was first described in 1962 by Lindquist & Meeuwisse (4) and independently by Liplane *et al* (3). The symptoms and signs are similar to those seen in disaccharide malabsorption with diarrhoea as the main problem. In the patients published up to now it has been fairly easy to establish the diagnosis as soon as the possibility of GGM has been thought of. Simple oral tolerance tests with various mono- and disaccharides with assay of sugars in blood and faeces have given a clear and diagnostic pattern (5). In a patient studied by us the findings in such simple tolerance tests did not allow definite diagnostic conclusions. For this reason and because only 20 cases have been published earlier including siblings who have died without a diagnosis of GGM but in whom a retrospective diagnosis has been established (6) we want to describe briefly the findings in our patient. Our report may illustrate that diagnostic problems do occur in GGM.

## CASE REPORT

A girl was born on January 19 1965. The parents were healthy and unrelated and as far as

could be found out not related to the family with GGM described from a different part of Sweden. (6). Birth weight 3130 g. Immediate neonatal development was unevenful. Diarrhoea started on the fourth day and soon became profuse and watery. The patient was referred to the Pediatric Department where treatment was started with antibiotics etc and iv infusions of Ringer-glucose solutions plasma and bicarbonate. Some improvement occurred of the general condition but the patient continued to have diarrhoea. Dietary changes with the institution of a lactose free formula did not radically improve the condition of the patient. There was nearly no weight gain during the first three months of life. Later a slow but steady weight gain occurred. All laboratory procedures failed to give a definite diagnosis for reasons discussed below. Since GGM was suspected a dietary formula with fructose as the only carbohydrate was instituted at age 4 months. This gave a radical improvement. The diarrhoea ceased the general condition of the patient improved and the weight increased. On a diet as formulated by Meeuwisse & Melin (5) the development of the patient has since then been normal.

Fig 1 outlines the growth of the patient. Fig 2 shows the appearance at age 4 months before the institution of the fructose formula.

*Laboratory tests*

Assay of serum electrolytes demonstrated that the patient suffered from hyperosmolar dehydration when the diarrhoea was severe. Small amounts of glucose were found in the urine and faeces on several occasions. pH in the watery stools was 5.0-5.5. All other routine laboratory tests were normal. On glucose galactose and lactose tolerance tests performed during the first months of life only very minute changes of blood glucose (glucose oxidase method commercial kit AB KABI Stockholm) and

\* Present address: Department of Clinical Chemistry University Hospital Lund Sweden

## CASE REPORT

# PROTEIN LOSING GASTROENTEROPATHY RESPONDING TO CORTICOSTEROID TREATMENT

EMANUEL LEBENTHAL, MIRIAM GAIFMAN and MENACHEM NITZAN

From the Department of Pediatrics, Beikson Medical Center, Tel Aviv University Medical School, Petach Tikva, Israel

Gastrointestinal protein loss is associated with many disease entities.

Allergic gastroenteropathy as a cause of excessive gastrointestinal protein loss was first described by Waldman *et al* in 1967 (20). The main abnormalities noted were hypoalbuminemia, hypogammaglobulinemia, anemia, eosinophilia, edema, growth retardation and gastrointestinal protein loss. The patients responded well to corticosteroids and to milk free diet.

We present here a case with similar features responding to corticosteroid treatment but without response to dietary elimination of milk.

### Case Report

A 10 year-old Sephardic boy was admitted at the age of 3 years to another hospital with suspected Celiac disease and was found to have protein losing gastroenteropathy with growth retardation. There was no family history of diarrhea or edema.

At 4 years of age he was first admitted to this department because of diarrhea, recurrent vomiting, abdominal pain, edema and growth retardation. His weight and height were in the 50th percentile for age. 3 years blood pressure was 90/60. He had generalized edema, the liver was palpable 4 cm below the right costal margin. Results of the examination was otherwise negative.

Laboratory find: Repeated normal serum were normal. The hemoglobin level ranged from 8.8 g/100 ml to 9.6 g/100 ml. The white cell count was between 7400-16100 with a high total eosinophil count of 800-2000 per mm<sup>3</sup>. The most remarkable laboratory

finding was a very low level of plasma proteins with a total of 12 g/100 ml, albumin 0.8 g/100 ml, globulin 1.4 g/100 ml. Quantitative immunoglobulins were as follows: IgG 540 mg/100 ml, IgM 40 mg/100 ml, IgA 52 mg/100 ml. The level of ceruloplasmin—0.028-0.100 density units (normal 0.100-0.200). Serum copper was from 23 µg/100 ml to 64 µg/100 ml. The serum iron ranged between 33-60 µg/100 ml. Serum calcium levels were between 6.8-8.3 mg/100 ml.

The following laboratory tests were negative or normal: Erythrocyte sedimentation rate, platelet counts, bleeding and coagulation times, prothrombin time, fibrinogen, protein bound iodine, BUN, serum electrolytes, fasting blood sugar, blood total lipids, cholesterol, phosphates, alkaline phosphatase, blood bilirubin, concentration, bromsulphalein retention tests, tapex erythrocytoma, cell preparation and karyotype test.

Stool examinations: Stools contained no occult blood, parasites or pathogenic organisms. Repeated D-xylose absorption tests and 72 hour stool collections for fat were within normal limits. Of an oral dose of 1 labeled tritolein 0.9% was excreted in the stools (normal <5). Vitamin A loading test showed a rise of serum Vitamin A from 116 to 300 µg/100 ml (normal). Sweat electrolytes were within normal limits. Glucose, galactose, sucrose and lactose absorption tests were normal.

On sigmoidoscopy the bowel had a normal appearance. An X-ray series of the gastrointestinal tract showed a normal esophagus and stomach, coarse mucosal folds and rapid flow in the small intestine. Barium enema showed no abnormalities. A chest roentgenogram was within normal limits. X-ray for determination of skeletal bone age revealed 4 years retardation.

Two per oral jejunal biopsies were taken with a Rubin tube, the first at the age of 7 years, the second

## CONCLUSIONS

The diagnosis of GGM now is definitely established in our patient. Our findings with oral tolerance tests illustrate that a diagnosis can not always be made as easily as in earlier published patients even when the patient does not have diarrhoea. Meeuwisse & Melin (5) state that 'The results of the sugar loading tests and of the dietetic trial with a formula, in which fructose is the only carbohydrate show that during the first months of life a guaranteed diagnosis can be made without further investigations'. This statement is applicable also to our patient if it is stressed that the dietetic trial was of more definite diagnostic value than the sugar loading tests.

## REFERENCES

- 1 Brante G. Anthronometodik för kolhydratbestämningar i det kliniska laboratoriet. *Svensk läkartidn* 49: 2516 1952
- 2 Heyrovsky A. A new method for the determination of inulin in plasma and urine. *Clin Chem Acta* 1: 470 1956
- 3 Laplane R, Polonovski C, Etienne M, Debry P, Lods J C & Pissarro B. L'intolérance au sucres a transfert intestinal actif. Ses rapports avec l'intolérance au lactose et le syndrome coeliaque. *Arch Franc Pédiat* 19: 895 1962
- 4 Lindquist B & Meeuwisse G W. Chronic diarrhoea caused by monosaccharide malabsorption. *Acta Paediat Scand* 51: 674 1962
- 5 Meeuwisse G W & Melin K. Glucose galactose malabsorption. A clinical study of 6 cases. *Acta Paediat Scand Suppl* 188: 3 1969
- 6 Melin K & Meeuwisse G W. Glucose galactose malabsorption. A genetic study. *Acta Paediat Scand Suppl* 188: 19 1969

Submitted Aug. 29 1969

Accepted Oct. 3 1969

(K. K.) Dept. of Paediatrics  
Centrallasarettet  
631 88 Eskilstuna  
Sweden

Key words: Glucose galactose malabsorption; diagnostic problems

from 1.4 to 2.3 g/100 ml (Fig. 2). The hemoglobin level increased to 12.2 g/100 ml and the total eosinophil count became normal. After 2 months of treatment with Metacorten the daily doses were decreased gradually to 7.5 mg Metacorten per day and he was discharged from the hospital on this daily dosage.

Two months later he was readmitted because of reappearance of generalized edema and hypoproteinemias. It was found that the child had not received any medication at home during this period. He was started on 30 mg Metacorten per day. Within 2 weeks the edema had again disappeared. The total serum proteins increased from 2.9 to 6.8 g/100 ml. Albumin rose from 1.1 to 3.7 g/100 ml, globulins from 1.8 to 3.1 g/100 ml. This pattern of the recurrence of edema and hypoproteinemias with cessation of the treatment with Metacorten and prompt improvement after reinstitution of therapy was noted several times for the next three years (Fig. 2). During those periods when he was not receiving Metacorten several therapeutic trials were attempted with gluten free, milk free and milk and gluten free diets. Each trial lasted 2-3 months and all were unsuccessful. The child remained edematous and hypoproteinemias during these treatments and improved only upon administration of Metacorten (Fig. 2).

There has been no improvement in the rate of growth during the 4 years of Metacorten treatment. At the age of 10 years he is at the 50th percentile for the age of 5 years.

## DISCUSSION

The clinical picture, the two normal intestinal biopsies, the gastrointestinal radiological findings and the normal findings of the sigmoidoscopy led us to exclude the following causes of protein losing enteropathy: giant hypertrophy of the gastric rugae (3), ulcerative colitis and regional enteritis (14), Whipple's intestinal lipodystrophy (8), gastrointestinal cancer (7), sprue (9) and constrictive pericarditis (4). A common cause of severe protein loss in

infancy and childhood is intestinal lymphangiectasia (6, 13, 19). In this condition the small intestine histology shows dilated lymphatic channels in the mucosa of the small intestine, there is fat malabsorption and there is no response to corticosteroids. Since our case presented none of the above phenomena and furthermore showed a clear response to corticosteroids, the diagnosis of lymphangiectasia can be excluded.

Another common cause in childhood is celiac disease (10, 17). In our patient there was no steatorrhea or other evidence of malabsorption; the biopsy was normal and there was no response to a gluten free diet. We also excluded agammaglobulinemia (6, 18) which may be associated with disorders of the gastrointestinal tract with excessive gastrointestinal protein loss.

Transient protein loss has also been described with hypocupremia and hypoferrremic anemia and eosinophilia (12, 15, 16). This group appears to represent several entities. In our case, however, the gastrointestinal protein loss is a constant feature and did not change when the anemia had improved.

The criteria given by Waldmann *et al.* (20) in their report on a number of cases included edema, extreme hypoproteinemias, anemia, eosinophilia, growth retardation and gastrointestinal protein loss which was corrected by the administration of corticosteroids and milk free diet. They proposed to call this syndrome allergic gastroenteropathy. Our case did not respond to several trials with milk free diet and therefore we cannot assume milk allergy to be the cause of the protein loss. It does however have features similar to those seen in the cases reported by Waldmann *et al.* (20) and it is possible that the cause of the disease is an allergy to an unknown substance.

## SUMMARY

A 10-year-old boy suffering from generalized edema, diarrhea, recurrent vomiting, abdominal pain and growth retardation was found

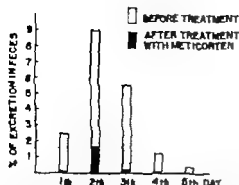


Fig 1 Excretion of  $^{125}\text{I}$  labeled albumin in the gastrointestinal tract before and after treatment with Meticorten (Prednisone)

at the age of 9  $\frac{1}{2}$  years. Both were done when he had been without corticosteroids for at least 2 months and was suffering from generalized edema and diarrhea. Both biopsy specimens were of normal appearance.

#### Studies of the protein loss

The loss of plasma proteins into the gastrointestinal tract was estimated by the use of  $^{125}\text{I}$  polyvinylpyrrolidone ( $^{125}\text{I}$  PVP) (4, 10, 17) and  $^{125}\text{I}$  labeled albumin (1, 2, 13). Each time he was given 12  $\mu\text{Ci}$  of the radioactive macromolecule intravenously and the fraction of the injected dose that appeared in the stools passed during the 5 days was determined. The methods for the assay of radioactivity in the stools have previously been described (5).

1  $^{125}\text{I}$  PVP revealed protein loss in stools of 11.7% of material (normal up to 1.5% in stools).

2  $^{125}\text{I}$  labeled albumin with amberlite showed 18.4% of the material in stools (normal up to 2%). Plasma albumin pool was very low—9.27 g (normal control with similar weight—42.3 g). Albumin half life 185 days (normal control 77 days). The daily turnover—13% (normal control—3–4%).

3  $^{125}\text{I}$ -labeled albumin test was repeated during corticosteroid treatment and only 2% was excreted in stools (Fig 1).

#### Therapeutic trials

A high protein diet (4 g per kg) was given for 5 months and was supplemented with 12.5 g salt free albumin and plasma. On this regime only a temporary increase was achieved in the serum protein level with no clinical improvement.

At this stage 5 months following his admission he was started on 20 mg Meticorten (Prednisone) per day in divided doses. Within 3 weeks a marked improvement was noted manifested by disappearance of the edema and diarrhea. The total proteins in the serum increased from 2.2 to 4.2 g/100 ml, globulins

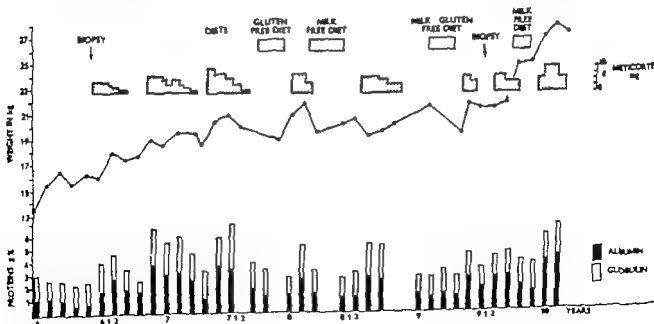


Fig 2 Response of serum proteins to corticosteroids and milk and gluten free diets

## CASE REPORT

### EXTENDED HEMODIALYSIS IN AN INFANT

CARL M. GRUSHFELD, RICHARD N. FINE and QUENTIN STILES

*From the Renal and Thoracic Surgery Divisions, Children's Hospital of Los Angeles and the Departments of Pediatrics and Surgery, University of Southern California School of Medicine, Los Angeles, California, U.S.A.*

Hemodialysis in infants and small children has been used primarily as treatment for acute renal failure (6, 7) and poisoning (5, 9). To our knowledge there is only one published report of extended hemodialysis for chronic renal failure in a child less than 3 years of age (10). In addition, 2 children between 2 and 3 years of age have been dialyzed for several months while awaiting transplantation (4). The present report describes extended hemodialysis in an 18-month-old infant during a 6 month period while awaiting cadaveric renal transplantation.

## METHODS

**Cannulation.** The teflon-silastic arteriovenous cannula described by Quinson *et al.* (8) was used. Under light general anesthesia the superficial femoral artery and saphenous vein were cannulated with no. 14 teflon type

**Delivery system.** A miniature artificial kidney system (MAKS No. 900 Bio Systems Inc. Santa Monica, California) was used to deliver the dialysate solution. The delivery system mixed tap water with a commercially prepared solution to the following concentrations: calcium 2.4-3.0 mEq/l, dextrose 700 mg/100 ml, magnesium 1.5 mEq/l, sodium 134 mEq/l, potassium 1 or 2 mEq/l, acetate 38 mEq/l and chloride 100 mEq/l. This fluid delivery system contains a positive displacement effluent pump which allows negative pressure adjustments from 0 to minus 450 mm Hg to facilitate ultrafiltration.

**Dialyzer.** One layer of a specially built 1/2 inch dialyzer with a total surface area of 0.5 m<sup>2</sup> was used for the first 8 dialyses. Subsequently 1 layer of a standard dialyzer was used for all dialyses. The fluid

volume of 1 layer of a standard dialyzer with 8 foot arterial and 7 foot venous blood tubing is approximately 150 ml.

**Technique of dialysis.** The blood tubing and dialyzer were primed with 150 ml of 0.9% saline to which 1500 units of aqueous heparin sodium/1000 ml were added. Depending upon the pre-dialysis blood pressure and weight 75 to 150 ml of the priming solution was administered to the patient at the start of dialysis. An additional 1000 units of aqueous heparin sodium were infused via the heparin line attached to the arterial blood tubing at the start of each dialysis. The clotting time of the venous blood outflow from the dialyzer was monitored every half hour and 500-1000 units of aqueous heparin sodium were administered every 1 to 2 hours to maintain the clotting time between 30 and 60 min.

## CASE REPORT

M.B. (B.D. 3/3/67), a Caucasian female (Fig. 1) was admitted to Children's Hospital of Los Angeles on 7/29/68 at 18 months of age. She was the product of a full term pregnancy and uncomplicated delivery. Her growth and development were normal. At age 15 months a right flank mass was noted. The right kidney was not visualized with intravenous pyelography. Exploratory laparotomy on 7/6/68 revealed a multicystic right kidney and a right nephrectomy was performed. During the procedure the left renal vein was inadvertently interrupted necessitating anastomosis with the inferior vena cava. The left renal artery was clamped for approximately 1 1/2 hours during the latter procedure. Postoperatively the patient was afebrile.

She was transferred elsewhere and the clinical diagnosis of acute renal failure due to acute tubular necrosis was made. The daily urine output was 60 to 90 ml. She was placed on a Franklin diet (3) and fluid intake was restricted to 10 ounces per day. Her



to have severe gastrointestinal protein loss with hypoalbuminemia, hypogammaglobulinemia and transient anemia and eosinophilia without malabsorption or gastrointestinal structural abnormalities. He responded well to corticosteroid treatment but there was no response to gluten free or milk free diet. Comparison with similar cases reported in the literature raises the possibility that this is a case of allergic gastroenteropathy due to an undetermined cause.

## REFERENCES

- 1 Bruman A & Rothschild M A. The rate of intravascular equilibration of intravenously administered  $^{125}$ I-labelled albumin in various body sites. *J Lab Clin Med* 48: 20 1956.
- 2 Berson S A, Schreiber S S & Post J. Tracer experiments with  $^{125}$ I-labelled human serum albumin. Distribution and degradation studies. *J Clin Invest* 32: 746 1953.
- 3 Citrin Y, Sterlme K & Halsted J A. Mechanism of hypoproteinemia associated with giant hypertrophy of gastric mucosa. *New Eng J Med* 257: 906 1957.
- 4 Davidson J D, Waldmann T A, Goodman D S & Gordon R S Jr. Protein losing gastroenteropathy in congestive heart failure. *Lancet* i: 899 1961.
- 5 Gordon R S Jr. Enterative enteropathy: abnormal permeability of gastrointestinal tract demonstrable with labelled PVP. *Lancet* i: 325 1959.
- 6 Holman H, Nickel W F Jr & Skeneer M H. Hypoproteinemia indicating intestinal lesions and possibly due to excessive serum protein loss into intestine. *Am J Med* 27: 963 1959.
- 7 Jarnum S & Schwartz M. Hypoalbuminemia in gastric carcinoma. *Gastroenterology* 38: 769 1960.
- 8 Laster L, Waldmann T A, Fenster L F & Singleton J W. Reversible enteric protein loss in Whipple's disease. *Gastroenterology* 42: 762 1962.
- 9 Parkins R A. Protein losing enteropathy in sprue syndrome. *Lancet* ii: 1366 1960.
- 10 Rotem Y & Czerniak P. Gastrointestinal protein leakage in celiac disease as studied by labeled PVP. *Am J Dis Child* 107: 38 1964.
- 11 Salazar De Sousa J, Gattaro O, Cunha A & Araujo J. Association of nephrotic syndrome with intestinal lymphangectasia. *Arch Dis Child* 43: 245 1968.
- 12 Schubert W A & Lahey M E. Copper and protein depletion complicating hypoproteinemic state of infancy. *Pediatrics* 24: 710 1959.
- 13 Schwartz M & Jarnum S. Protein losing gastroenteropathy: hypoproteinemia due to enteric intestinal protein loss of varying aetiology diagnosed by means of  $^{125}$ I-albumin. *Dansk Med Bull* 8: 1 1961.
- 14 Steinfeld J L, Davidson J D, Gordon R S Jr & Greene F E. Mechanism of hypoproteinemia in patients with regional enteritis and ulcerative colitis. *Am J Med* 29: 405 1960.
- 15 Sturezon P & Brubaker C. Copper deficiency in infants: syndrome characterized by hypochromic iron deficiency anemia and hypoproteinemia. *Am J Dis Child* 97: 254 1956.
- 16 Ulstrom R A, Smith N J & Heimlich E M. Transient dysproteinemia in infants: new syndrome? Clinical studies. *Am J Dis Child* 97: 219 1956.
- 17 Troupel S, Acar J, Renault H, Desbrosse G & Cottan R. Enteropathie avec perte de protéines et stéatorrhée: étude par le PVP $^{125}$  et la tréhalose  $^{125}$  action du régime sans gluten. *Bull Soc Med Hop Paris* 76: 261 1960.
- 18 Waldmann T A, Steinfeld J L, Dutcher T F, Davidson J D & Gordon R S Jr. Role of gastrointestinal system in idiopathic hypoproteinemia. *Gastroenterology* 41: 197 1961.
- 19 Waldmann T A & Laster L. Abnormalities of albumin metabolism in patients with hypogammaglobulinemia. *J Clin Invest* 43: 1025 1964.
- 20 Waldmann T A, Woosner R D, Laster L and Gordon R S Jr. Allergic gastroenteropathy: a cause of excessive gastrointestinal protein loss. *New Eng J Med* 276: 761 1967.

Submitted July 28 1969

Accepted Oct 1 1969

(E. L.) Dept of Paediatrics  
Beilinson Medical Center  
P.O.B. 85  
Petah Tikva  
Israel

**Key words:** Gastroenteropathy, protein loss, corticosteroid treatment.

## CASE REPORT

### EXTENDED HEMODIALYSIS IN AN INFANT

CARL M. GRUSHKIN, RICHARD N. FINE and QUENTIN STILES

*From the Renal and Thoracic Surgery Divisions, Children's Hospital of Los Angeles and the Departments of Pediatrics and Surgery, University of Southern California School of Medicine, Los Angeles, California, U.S.A.*

Hemodialysis in infants and small children has been used primarily as treatment for acute renal failure (6, 7) and poisoning (5, 9). To our knowledge there is only one published report of extended hemodialysis for chronic renal failure in a child less than 3 years of age (10). In addition 2 children between 2 and 3 years of age have been dialyzed for several months while awaiting transplantation (4). The present report describes extended hemodialysis in an 18-month-old infant during a 6 month period while awaiting cadaveric renal transplantation.

## METHODS

**Cannulation.** The iliofemoral arteriovenous cannula described by Quenton *et al.* (8) was used. Under light general anesthesia the superficial femoral artery and superficial vein were cannulized with no. 14 teflon tips.

**Delivery system.** A miniature artificial kidney system (NAKS No. 900 Bio-System, Inc. Santa Monica, California) was used to deliver the dialysis solution. The delivery system mixed tap water with a commercially prepared solution to the following concentrations: calcium 2.4-3.0 mEq/l, dextrose 80 mg/100 ml, magnesium 1.5 mEq/l, sodium 134 mEq/l, potassium 1 or 2 mEq/l, acetate 38 mEq/l and chloride 100 mEq/l. This fluid delivery system contains a positive displacement effluent pump which allows negative pressure adjustments from 0 to minus 450 mm Hg to facilitate ultrafiltration.

**Dialyzer.** One layer of a specially built / Kd dialyzer with total surface area of 0.5 m<sup>2</sup> was used for the first 8 dialyses. Subsequently 1 layer of a standard dialyzer was used for all dialyses. The fluid

volume of 1 layer of a standard dialyzer with 8 foot arterial and 7 foot venous blood tubing is approximately 150 ml.

**Technique of dialysis.** The blood tubing and dialyzer were primed with 150 ml of 0.9 N saline to which 1500 units of aqueous heparin sodium/1000 ml were added. Depending upon the pre-dialysis blood pressure and weight 75 to 150 ml of the priming solution was administered to the patient at the start of dialysis. An additional 1000 units of aqueous heparin sodium were infused via the heparin line attached to the arterial blood tubing at the start of each dialysis. The clotting time of the venous blood outflow from the dialyzer was monitored every half hour and 500-1000 units of aqueous heparin sodium were administered every 1 to 2 hours to maintain the clotting time between 30 and 60 min.

## CASE REPORT

M.B. (B.D. 3367), a Caucasian female (Fig. 1) was admitted to Children's Hospital of Los Angeles on 11/9/68 at 18 months of age. She was the product of a full term pregnancy and uncomplicated delivery. Her growth and development were normal. At age 15 months a right flank mass was noted. The right kidney was not visualized with intravenous pyelography. Exploratory laparotomy on 7/6/68 revealed a multicystic right kidney and a right nephrectomy was performed. During the procedure the left renal vein was inadvertently interrupted necessitating reanastomosis with the inferior vena cava. The left renal artery was clamped for approximately 1 1/2 hours during the latter procedure. Postoperatively the patient was anemic.

She was transferred elsewhere and the clinical diagnosis of acute renal failure due to acute tubular necrosis was made. The daily urine output was 60 to 90 ml. She was placed on a Franklin diet (3) and fluid intake was restricted to 10 ounces per day. Her

to have severe gastrointestinal protein loss with hypoalbuminemia, hypogammaglobulinemia and transient anemia and eosinophilia without malabsorption or gastrointestinal structural abnormalities. He responded well to corticosteroid treatment but there was no response to gluten free or milk free diet. Comparison with similar cases reported in the literature raises the possibility that this is a case of allergic gastroenteropathy due to an undetermined cause.

## REFERENCES

- 1 Bauman A & Rothschild M A The rate of intravascular equilibration of intravenously administered  $I^{125}$  labelled albumin in various body sites *J Lab Clin Med* 48 20 1956
- 2 Berson S A Schreiber S S & Post J Tracer experiments with  $I^{125}$  labelled human serum albumin. Distribution and degradation studies *J Clin Invest* 32 746 1953
- 3 Citrin Y Sterling K & Halsted J A Mechanism of hypoproteinemia associated with giant hypertrophy of gastric mucosa *New Eng J Med* 257 906 1957
- 4 Davidson J D Waldmann T A Goodman D S & Gordon R S Jr Protein losing gastroenteropathy in congestive heart failure *Lancet* i 899 1961
- 5 Gordon R S Jr Exudative enteropathy: abnormal permeability of gastrointestinal tract demonstrable with labelled PVP *Lancet* i 325 1959
- 6 Holman H Nickel W F Jr & Slesinger M H Hypoproteinemia antecedent intestinal lesions and possibly due to excessive serum protein loss into intestine *Am J Med* 27 963 1959
- 7 Jarnum S & Schwartz M Hypoalbuminemia in gastric carcinoma *Gastroenterology* 38 769 1960
- 8 Laster L Waldmann T A Fenster L F & Singleton J W Reversible enteric protein loss in Whipple's disease *Gastroenterology* 42 762 1962
- 9 Parkins R A Protein losing enteropathy in sprue syndrome *Lancet* ii 1366 1960
- 10 Rotem Y & Czerniak P Gastrointestinal

- protein leakage in celiac disease as studied by labeled PVP *Am J Dis Child* 107 58 1964
- 11 Salazar De Sousa J Guerrero B Cunha A & Arrujo J Association of nephrotic syndrome with intestinal lymphangiectasia *Arch Dis Child* 43 245 1968
  - 12 Schubert W K & Labey M E Copper and protein depletion complicating hypoproteinemic of infancy *Pediatrics* 24 710 1959
  - 13 Schwartz M & Jarnum S Protein losing gastroenteropathy hypoproteinemia due to gastrointestinal protein loss of varying aetiology diagnosed by means of  $^{125}I$  albumin *Danish Med Bull* 8 1 1961
  - 14 Steinfeld J L Davidson J D Gordon R S Jr & Greene F E Mechanism of hypoproteinemia in patients with regional enteritis and ulcerative colitis *Am J Med* 29 405 1960
  - 15 Sturgeon P & Brubaker C Copper deficiency in infants syndrome characterized by hypocupremia iron deficiency anemia and hypoproteinemia *Am J Dis Child* 92 254 1956
  - 16 Ulstrom R A Smith N J & Heimlich E M Transient dysproteinemia in infants new syndrome I Clinical studies *Am J Dis Child* 97 219 1956
  - 17 Troupeu S Acar J Rensault H Desboques G & Cottan R Enteropathie avec perte de protéines et séatorrhée étude par le PVP  $I^{125}$  III la tolérance à l'action du régime sans gluten *Bull Soc Med Hop Paris* 76 261 1960
  - 18 Waldmann T A Steinfeld J L Dutcher T F Davidson J D & Gordon R S Jr Role of gastrointestinal system in idiopathic hypoproteinemia *Gastroenterology* 41 197 1961
  - 19 Waldmann T A & Laster L Abnormalities of albumin metabolism in patients with hypogammaglobulinemia *J Clin Invest* 43 1025 1964
  - 20 Waldmann T A Wochner R B Laster L and Gordon R S Jr Allergic gastroenteropathy a cause of excessive gastrointestinal protein loss *New Eng J Med* 276 761 1967

Submitted July 28 1969

Accepted Oct 1 1969

(E L) Dept of Paediatrics  
Beilinson Medical Center  
P O B 85  
Petah Tiqva  
Israel

**Key words** Gastroenteropathy protein loss corticosteroid treatment

## CASE REPORT

### EXTENDED HEMODIALYSIS IN AN INFANT

CARL H. KRUSHKIN, RICHARD N. FINE and QUENTIN STILES

From the Renal and Thoracic Surgery Divisions, Children's Hospital of Los Angeles and the Departments of Pediatrics and Surgery, University of Southern California School of Medicine, Los Angeles, California, U.S.A.

Hemodialysis in infants and small children has been used primarily as treatment for acute renal failure (6, 7) and poisoning (8, 9). To our knowledge there is only one published report of extended hemodialysis for chronic renal failure in a child less than 3 years of age (10). In addition, 2 children between 2 and 3 years of age have been dialyzed for several months while awaiting transplantation (4). The present report describes extended hemodialysis in an 18-month-old infant during a 6 month period while awaiting cadaveric renal transplantation.

## METHODS

**Cannulation.** The left-iliac-arteriovenous circuit described by Quenton *et al.* (3) was used. Under light general anesthesia the superficial femoral artery and saphenous vein were cannulated with no. 14 teflon tips.

**Delivery system.** A miniature artificial kidney system (MAKS No. 900 Bio-Syncron, Inc., Santa Monica, California) was used to deliver the dialysate solution. The delivery system served tap water with a commercially prepared solution to the following composition: calcium 2.4-3.0 mEq/l, dextrose 200 mg/100 ml, magnesium 1.5 mEq/l, sodium 134 mEq/l, potassium 3 or 4 mEq/l, acetate 33 mEq/l and chloride 100 mEq/l. This fluid delivery system contains a positive displacement efficient pump which allows negative pressure adjustments from 0 to minus 450 mm Hg to facilitate ultrafiltration.

**Fluid.** One layer of a specially built 1/2 inch dialyzer with a total surface area of 0.5 m<sup>2</sup> was used for the first 8 dialyses. Subsequently 1 layer of a standard dialyzer was used for all dialyses. The fluid

volume of 1 layer of a standard dialyzer with 8 foot arterial and 7 foot venous blood tubing is approximately 150 ml.

**Technique of dialysis.** The blood tubing and dialyzer were primed with 150 ml of 0.9% saline to which 1500 units of aqueous heparin sodium/1000 ml were added. Depending upon the pre-dialysis blood pressure and weight 75 to 150 ml of the priming solution was administered to the patient at the start of dialysis. An additional 1000 units of aqueous heparin sodium were infused via the heparin line attached to the arterial blood tubing at the start of each dialysis. The clotting time of the venous blood outflow from the dialyzer was monitored every half hour and 500-1000 units of aqueous heparin sodium were administered every 1 to 2 hours to maintain the clotting time between 30 and 60 min.

## CASE REPORT

M.B. (B.D. 3367), a Caucasian female (Fig. 1) was admitted to Children's Hospital of Los Angeles on 22-9-68 at 18 months of age. She was the product of a full term pregnancy and uncomplicated delivery. Her growth and development were normal. At age 15 months a right flank mass was noted. The right kidney was not visualized with intravenous pyelography. Exploratory laparotomy on 7-6-68 revealed a multicystic right kidney and a right nephrectomy was performed. During the procedure the left renal vein was inadvertently interrupted, accumulating anastomosis with the inferior vena cava. The left renal artery was clamped for approximately 1 1/2 hours during the latter procedure. Postoperatively the patient was anemic.

She was transferred elsewhere and the clinical diagnosis of acute renal failure due to acute tubular necrosis was made. The daily urine output was 60 to 90 ml. She was placed on a Franklin diet (1) and fluid intake was restricted to 10 ounces per day. Her

to have severe gastrointestinal protein loss with hypalbuminemia, hypogammaglobulinemia and transient anemia and eosinophilia without malabsorption or gastrointestinal structural abnormalities. He responded well to corticosteroid treatment but there was no response to gluten free or milk free diet. Comparison with similar cases reported in the literature raises the possibility that this is a case of allergic gastroenteropathy due to an undetermined cause.

## REFERENCES

- Bauman A & Rothchild M A. The rate of intravascular equilibration of intravenously administered  $I^{125}$  labelled albumin in various body sites. *J Lab Clin Med* 48: 20, 1956.
- Berson S A, Schreiber S S & Post J. Tracer experiments with  $I^{125}$  labelled human serum albumin. Distribution and degradation studies. *J Clin Invest* 32: 746, 1953.
- Citrin Y, Sterling K & Hulsed J A. Mechanism of hypoproteinemia associated with giant hypertrophy of gastric mucosa. *New Eng J Med* 257: 906, 1957.
- Davidson J D, Waldmann T A, Goodman E S & Gordon R S Jr. Protein losing gastroenteropathy in congestive heart failure. *Lancet* 1: 899, 1961.
- Gordon R S Jr. Exudative enteropathy: abnormal permeability of gastrointestinal tract demonstrable with labelled PVP. *Lancet* 1: 325, 1959.
- Holman H, Nickel W F Jr & Skensenger M H. Hypoproteinemia indicating intestinal lesions and possibly due to excessive serum protein loss into intestine. *Am J Med* 27: 963, 1959.
- Jarman S & Schwartz M. Hypoalbuminemia in gastric carcinoma. *Gastroenterology* 38: 769, 1960.
- Laster L, Waldmann T A, Fenster L E & Singleton J W. Reversible enteric protein loss in Whipple's disease. *Gastroenterology* 42: 762, 1962.
- Parkins R A. Protein losing enteropathy in sprue syndrome. *Lancet* II: 1366, 1960.
- Rotem Y & Czerniak P. Gastrointestinal protein leakage in celiac disease as studied by labeled PVP. *Am J Dis Child* 107: 53, 1964.
- Salazar De Sousa J, Guerrero O, Canha A & Araujo J. Association of nephrotic syndrome with intestinal lymphangiectasia. *Arch Dis Child* 43: 245, 1968.
- Schubert W K & Lahey M E. Copper and protein depletion complicating hypoproteinemic state of infancy. *Pediatrics* 74: 710, 1959.
- Schwartz M & Jarman S. Protein losing gastroenteropathy: hypoproteinemia due to gastrointestinal protein loss of varying aetiology, diagnosed by means of  $I^{125}$  albumin. *Dtsch Med Bull* 8: 1, 1961.
- Stemfeld J L, Davidson J D, Gordon R S Jr & Greene F E. Mechanism of hypoproteinemia in patients with regional enteritis and ulcerative colitis. *Am J Med* 29: 405, 1960.
- Sturgeon P & Brubaker C. Copper deficiency in infants: syndrome characterized by hypoproteinemia, iron deficiency anemia and hypoproteinemia. *Am J Dis Child* 92: 254, 1956.
- Ulstrom R A, Smith N J & Henrich E M. Transient dysproteinemia in infants: new syndrome. I. Clinical studies. *Am J Dis Child* 92: 219, 1956.
- Troupel S, Acar J, Renault H, Desboques G & Cottin R. Enteropathie avec perte de protéines et ataxorrhée: étude par le PVP  $I^{125}$  et la tréhalose  $I^{125}$ : action du régime sans gluten. *Bull Soc Méd Hop Paris* 76: 261, 1960.
- Waldmann T A, Stemfeld J L, Dutcher T F, Davidson J D & Gordon R S Jr. Role of gastrointestinal system in idiopathic hypoproteinemia. *Gastroenterology* 41: 197, 1961.
- Waldmann T A & Laster L. Abnormalities of albumin metabolism in patients with hypoxaemic malobolusmia. *J Clin Invest* 43: 1025, 1964.
- Waldmann T A, Wochner R D, Laster L and Gordon R S Jr. Allergic gastroenteropathy: a cause of excessive gastrointestinal protein loss. *New Eng J Med* 276: 761, 1967.

Submitted July 28, 1969

Accepted Oct. 1, 1969

(E. L.) Dept. of Paediatrics

Beilinson Medical Center

P.O.B. 95

Petah Tikva

Israel

Key words: Gastroenteropathy, protein loss, corticosteroid treatment.

only in the recumbent position obtained by palpation of the brachial artery were used. Ultrafiltration with weight loss produced an average decrease in the blood pressure of 25 mm Hg.

**Ultrafiltration.** Pre and post-dialysis weights are shown in the lower half of Fig. 2. The average weight loss during each 6 hours of dialysis was 1 pound. During the month of October the infant had persistent and increasing edema despite maximal ultrafiltration. The serum albumin was less than 2.5 g/100 ml. The intravenous administration of 25 ml of 25% salt poor albumin during 3 dialyses increased the serum albumin to greater than 3.0 g/100 ml and facilitated fluid removal. A decrease in the average post dialysis weight from 3 to 4 pounds then occurred and all edema disappeared.

**Anemia.** In general the pre dialysis hematocrit level of all children in our hemodialysis program is maintained at 18%. Sedimented red blood cells are given when the pre dialysis level is less than 18%. In a 20 pound infant approximately 20% of the blood volume is circulating in the dialyzer and tubing during dialysis. In order to minimize the effects of a technical mishap the pre-dialysis hematocrit of this patient was maintained at 22%. Approximately 300 ml per month of sedimented red blood cells were necessary to maintain the hematocrit at this level. Several transfusions were necessitated by complications which will be discussed later.

**Biochemical.** The average pre dialysis BUN, serum potassium and serum calcium are listed in Table 1. Despite pre-dialysis BUN levels of greater than 90 mg/100 ml the infant did not have clinical manifestations of uremia. During the second month of dialysis the pre-dialysis serum potassium occasionally exceeded 7 mEq/l. Avoidance of foods with high potassium content prevented hyperkalemia. Serum calcium levels were maintained within the normal range (8.5-10.5 mg/100 ml) without using supplemental calcium or Vitamin D.

**Diet.** During the first 1 1/2 months of hemo-

Table 1 Pre dialysis average value per month

	BUN (mg %)	Serum potassium (mEq/l)	Serum calcium (mg %)
October	111	4.1	7.6
November	51	5.9	9.6
December	63	4.9	9.1
January	112	6.1	9.4
February	94	5.2	8.4
March	75	5.0	8.5

dialysis the diet was not restricted. Fluid intake was limited to 12 ounces daily. Fluid retention, hypertension and occasional asymptomatic hyperkalemia (a greater than 7 mEq/l) occurred. Fluid intake was then limited to 8 ounces daily and foods high in potassium such as milk, potatoes, chocolate and orange juice were eliminated. A calorie count after 4 months of dialysis revealed an intake of 485 calories daily. A new diet was prescribed to increase the caloric intake to 1175 calories daily. However, a repeat calorie count after 5 1/2 months of dialysis revealed an intake of only 600 calories daily. Despite this caloric intake which is less than that recommended growth occurred (1).

**Growth.** During the 6 months of dialysis the patient grew 7 inches (Fig. 3). The rate of growth during this period was normal for a child of this age.

**Weight gain.** An indirect assessment of weight gain is shown in Fig. 2. Comparison of pre-dialysis weight versus pre-dialysis blood pressure shows that a direct relationship exists between fluid retention and increased blood pressure. Fluid removal during dialysis was followed by a decrease in blood pressure. During the November-March period of dialysis the post-dialysis weight increased from 18 to 20 pounds without alteration of the post-dialysis blood pressure. These observations suggest that the weight gain is "real" rather than a reflection of retained fluid.

**Complications.** Superficial skin infection which responded rapidly to oral antistaphylococcal antibiotics occurred on one occasion around the arterial exit site of the cannula.



Fig 1 Patient after 5 months of dialysis

condition failed to improve and between July 1 and September 20 1968 7 peritoneal dialyses were performed. Between dialyses she was maintained on a low potassium Franklin diet supplemental calcium vitamin D Amphojel® and limited fluid intake. She was transferred to Childrens Hospital of Los Angeles for hemodialysis and renal transplantation.

Physical examination upon admission revealed a debilitated infant with decreased muscle mass and subcutaneous tissue. Moderate edema of the face hands and feet was present. There were no signs of vitamin deficiency or bleeding. The blood pressure in the recumbent position was 130/90 mm Hg the pulse rate was 118 per minute and respiratory rate 30 per minute. The height was 29 inches and weight 19 and 1/2 pounds. There was a right nephrectomy scar and several small midline scars over the abdomen. The liver was not enlarged and there were no palpable abdominal masses. Radiographic examination of the chest showed infiltrates in the right middle and upper lobes. The heart size was normal. There was no radiographic evidence of rickets.

On admission the hemoglobin was 7.9 g/100 ml platelets 47 000/mm<sup>3</sup> blood urea nitrogen (BUN) 48 mg/100 ml serum creatinine 4.6 mg/100 ml serum sodium 150 mEq/l serum potassium 4.1 mEq/l serum calcium 7.5 mg/100 ml and serum albumin 2.8 g/100 ml.

A teflon plastic arteriovenous cannula was inserted on 23 9 68 and hemodialysis was begun on

24 9 68. Because of persistent vomiting an upper gastrointestinal series was obtained on 27 9 68. No barium was seen beyond the duodenum indicating a probable high small bowel obstruction. While preparing for surgical exploration a cadaveric kidney from a 48 hour old anencephalic male newborn became available. A renal transplant was performed and adhesions involving the small bowel were lysed. Within 30 min after completion of the vascular anastomosis the transplanted kidney became progressively darker in color. A diagnosis of hyperacute rejection was made and the kidney removed. On 11 10 68 a second laparotomy was performed because of persistent vomiting and fresh adhesions involving the small bowel were lysed. No further vomiting occurred. On 17 10 68 she was discharged without medications to return three weekly for hemodialysis.

## RESULTS AND COMMENTS

**Arteriovenous cannula** The superficial femoral artery and saphenous vein were of sufficient caliber to insert no. 14 teflon tips. With tips of this size, flow rates greater than 100 ml/min were obtained without the aid of a blood pump provided that systolic blood pressure was greater than 90 mm Hg. Williams described arteriovenous cannulation utilizing an artery and vein in the forearm of a 2 1/2 year old child (10). Flow rates through the specially built dialyzer averaged 40 ml/min. Fluid removal with this low flow rate was difficult and 2 peritoneal dialyses were necessary during a 6 week period to reduce edema.

**Blood pressure** Monthly average pre and post dialysis blood pressures are shown in the top half of Fig. 2. Systolic blood pressures

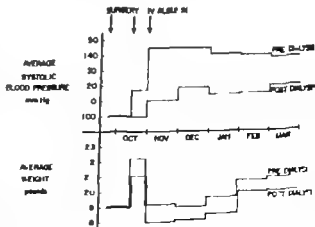


Fig 2 Effect of hemodialysis on blood pressure and weight

only in the recumbent position obtained by palpation of the brachial artery were used. Ultrafiltration with weight loss produced an average decrease in the blood pressure of 25 mm Hg.

**Ultrafiltration** Pre and post dialysis weights are shown in the lower half of Fig. 2. The average weight loss during each 6 hours of dialysis was 1 pound. During the month of October the infant had persistent and increasing edema despite maximal ultrafiltration. The serum albumin was less than 2.5 g/100 ml. The intravenous administration of 25 ml of 25% salt poor albumin solution during 3 dialyses increased the serum albumin to greater than 3.0 g/100 ml and facilitated fluid removal. A decrease in the average post-dialysis weight from 3 to 4 pounds then occurred and all edema disappeared.

**Anemia** In general the pre-dialysis hematocrit level of all children in our hemodialysis program is maintained at 18-22. Sedimented red blood cells are given when the pre-dialysis level is less than 18. In a 20 pound infant approximately 20% of the blood volume is circulating in the dialyzer and tubing during dialysis. In order to minimize the effects of a technical mishap the pre-dialysis hematocrit of this patient was maintained at 22. Approximately 300 ml per month of sedimented red blood cells were necessary to maintain the hematocrit at this level. Several transfusions were necessitated by complications which will be discussed later.

**Biochemical** The average pre-dialysis BUN, serum potassium and serum calcium are listed in Table 1. Despite pre-dialysis BUN levels of greater than 90 mg/100 ml the infant did not have clinical manifestations of uremia. During the second month of dialysis the pre-dialysis serum potassium occasionally exceeded 7 mEq/l. Avoidance of foods with high potassium content prevented hyperkalemia. Serum calcium levels were maintained within the normal range (8.5-10.5 mg/100 ml) without using supplemental calcium or Vitamin D.

**Diet** During the first 1 1/2 months of hemo-

Table 1 Pre dialysis average value per month

	BUN (mg )	Serum potassium (mEq/l)	Serum calcium (mg )
October	25	4.1	7.6
November	51	5.9	9.6
December	63	4.9	9.1
January	112	6.1	9.4
February	94	5.2	8.4
March	75	5.0	8.5

dialysis the diet was not restricted. Fluid intake was limited to 12 ounces daily. Fluid retention, hypertension and occasional asymptomatic hyperkalemia (K greater than 7 mEq/l) occurred. Fluid intake was then limited to 8 ounces daily and foods high in potassium such as milk, potatoes, chocolate and orange juice were eliminated. A calorie count after 4 months of dialysis revealed an intake of 485 calories daily. A new diet was prescribed to increase the caloric intake to 1175 calories daily. However a repeat calorie count after 5 1/2 months of dialysis revealed an intake of only 600 calories daily. Despite this caloric intake which is less than that recommended growth occurred (1).

**Growth** During the 6 months of dialysis the patient grew 3 inches (Fig. 3). The rate of growth during this period was normal for a child of this age.

**Weight gain** An indirect assessment of weight gain is shown in Fig. 2. Comparison of pre-dialysis weight versus pre-dialysis blood pressure shows that a direct relationship exists between fluid retention and increased blood pressure. Fluid removal during dialysis was followed by a decrease in blood pressure. During the November-March period of dialysis the post-dialysis weight increased from 18 to 20 pounds without alteration of the post-dialysis blood pressure. These observations suggest that the weight gain is real rather than a reflection of retained fluid.

**Complications** Superficial skin infection which responded rapidly to oral antistaphylococcal antibiotics occurred on one occasion around the arterial exit site of the cannula.





Fig 1 Patient after 5 months of dialysis

condition failed to improve and between July 1 and September 20 1968 7 peritoneal dialyses were performed. Between dialyses she was maintained on a low potassium Franklin diet supplemental calcium vitamin D Amphogel<sup>®</sup> and limited fluid intake. She was transferred to Childrens Hospital of Los Angeles for hemodialysis and renal transplantation.

Physical examination upon admission revealed a debilitated infant with decreased muscle mass and subcutaneous tissue. Moderate edema of the face hands and feet was present. There were no signs of vitamin deficiency or bleeding. The blood pressure in the recumbent position was 130/90 mm Hg the pulse rate was 118 per minute and respiratory rate 30 per minute. The height was 29 inches and weight 19 and 1/2 pounds. There was a right nephrectomy scar and several small midline scars over the abdomen. The liver was not enlarged and there were no palpable abdominal masses. Radiographic examination of the chest showed infiltrates in the right middle and upper lobes. The heart size was normal. There was no radiographic evidence of rickets.

On admission the hemoglobin was 7.9 g/100 ml platelets 47 000/mm<sup>3</sup> blood urea nitrogen (BUN) 48 mg/100 ml serum creatinine 4.6 mg/100 ml serum sodium 150 mEq/l serum potassium 4.1 mEq/l serum calcium 7.5 mg/100 ml and serum albumin 2.8 g/100 ml.

A teflon silastic arteriovenous cannula was inserted on 23/9/68 and hemodialysis was begun on

24/9/68. Because of persistent vomiting an upper gastrointestinal series was obtained on 27/9/68. No barium was seen beyond the duodenum indicating a probable high small bowel obstruction. While preparing for surgical exploration a cadaveric kidney from a 48 hour old anencephalic male newborn became available. A renal transplant was performed and adhesions involving the small bowel were lysed. Within 30 min after completion of the vascular anastomosis the transplanted kidney became progressively darker in color. A diagnosis of hyperacute rejection was made and the kidney removed. On 11/10/68 a second laparotomy was performed because of persistent vomiting and fresh adhesions involving the small bowel were lysed. No further vomiting occurred. On 17/10/68 she was discharged without indications to return thrice weekly for hemodialysis.

## RESULTS AND COMMENTS

**Arteriovenous cannula** The superficial femoral artery and saphenous vein were of sufficient caliber to insert no. 14 teflon tips. With tips of this size flow rates greater than 100 ml/min were obtained without the aid of a blood pump provided that systolic blood pressure was greater than 90 mm Hg. Williams described arteriovenous cannulation utilizing an artery and vein in the forearm of a 2 1/2 year old child (10). Flow rates through the specially built dialyzer averaged 40 ml/min. Fluid removal with this low flow rate was difficult and 2 peritoneal dialyses were necessary during a 6 week period to reduce edema.

**Blood pressure** Monthly average pre and post dialysis blood pressures are shown in the top half of Fig 2. Systolic blood pressures

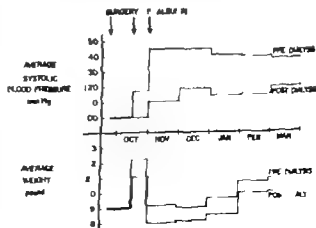


Fig 2 Effect of hemodialysis on blood pressure and weight

only in the recumbent position obtained by palpation of the brachial artery were used. Ultrafiltration with weight loss produced an average decrease in the blood pressure of 25 mm Hg.

Ultrafiltration Pre and post dialysis weights are shown in the lower half of Fig 2. The average weight loss during each 6 hours of dialysis was 1 pound. During the month of October the infant had persistent and increasing edema despite maximal ultrafiltration. The serum albumin was less than 2.5 g/100 ml. The intravenous administration of 25 ml of 25% salt poor albumin during 3 dialyses increased the serum albumin to greater than 3.0 g/100 ml and facilitated fluid removal. A decrease in the average post dialysis weight from 3 to 4 pounds then occurred and all edema disappeared.

**Anemia.** In general the pre dialysis hematocrit level of all children in our hemodialysis program is maintained at 18% (2). Sedimented red blood cells are given when the pre dialysis level is less than 18%. In a 20 pound infant approximately 20% of the blood volume is circulating in the dialyzer and tubing during dialysis. In order to minimize the effects of a technical mishap the pre dialysis hematocrit of this patient was maintained at 22%. Approximately 300 ml per month of sedimented red blood cells were necessary to maintain the hematocrit at this level. Several transfusions were necessitated by complications which will be discussed later.

**Biochemical.** The average pre dialysis BUN, serum potassium and serum calcium are listed in Table 1. Despite pre dialysis BUN levels of greater than 90 mg/100 ml the infant did not have clinical manifestations of uremia. During the second month of dialysis the pre dialysis serum potassium occasionally exceeded 7 mEq/l. Avoidance of foods with high potassium content prevented hyperkalemia. Serum calcium levels were maintained within the normal range (8.5-10.5 mg/100 ml) without using supplemental calcium or Vitamin D.

**Diet.** During the first 1½ months of hemo-

Table 1 Pre dialysis average value per month

	BUN (mg%)	Serum potassium (mEq/l)	Serum calcium (mg%)
October	25	4.1	7.6
November	31	5.9	9.6
December	63	4.9	9.1
January	112	6.1	9.4
February	94	5.2	8.4
March	75	5.0	8.3

dialysis the diet was not restricted. Fluid intake was limited to 12 ounces daily. Fluid retention, hypertension and occasional asymptomatic hyperkalemia (K greater than 7 mEq/l) occurred. Fluid intake was then limited to 8 ounces daily and foods high in potassium such as milk, potatoes, chocolate and orange juice were eliminated. A calorie count after 4 months of dialysis revealed an intake of 485 calories daily. A new diet was prescribed to increase the caloric intake to 1175 calories daily. However a repeat calorie count after 5½ months of dialysis revealed an intake of only 600 calories daily. Despite this caloric intake which is less than that recommended growth occurred (1).

**Growth.** During the 6 months of dialysis the patient grew 3 inches (Fig 3). The rate of growth during this period was normal for a child of this age.

**Weight gain.** An indirect assessment of weight gain is shown in Fig 2. Comparison of pre-dialysis weight versus pre-dialysis blood pressure shows that a direct relationship exists between fluid retention and increased blood pressure. Fluid removal during dialysis was followed by a decrease in blood pressure. During the November-March period of dialysis the post-dialysis weight increased from 18 to 20 pounds without alteration of the post dialysis blood pressure. These observations suggest that the weight gain is "real" rather than a reflection of retained fluid.

**Complications.** Superficial skin infection which responded rapidly to oral antistaphylococcal antibiotics occurred on one occasion around the arterial exit site of the cannula.

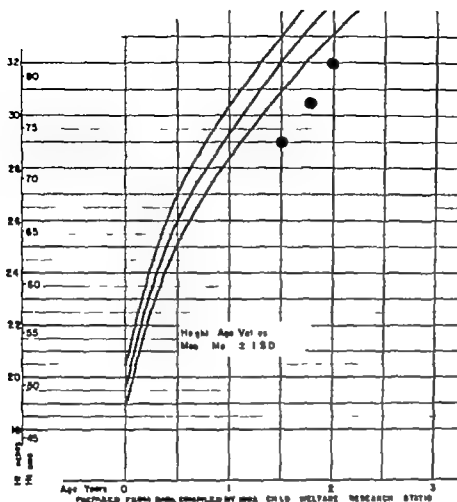


Fig 3 Growth chart

Clotting in the arteriovenous cannula occurred on 4 occasions. The clot was easily removed on the first 2 occasions. The third necessitated surgical revision with a new teflon tip placed in the artery and the fourth necessitated transfer of the cannula to the other leg. The initial cannula functioned for 5½ months. Blood transfusion to raise the hematocrit to 30% was necessary to prepare the patient for both surgical procedures. Clotting of the dialyzer occurred once and a transfusion was needed for replacement.

During three dialyses the systolic blood pressure fell rapidly to 60–70 mm Hg due to excessive ultrafiltration and fluid removal. Hypotension was easily and rapidly corrected by the administration of 0.9 N saline via the venous drip chamber.

**Clinical response.** On admission to the hospital the infant was hypotonic, did not sit with

out support or smile. After 2 months of dialysis she sat without support and after 4 months walked with support. Her affect improved and she began to smile and talk after 3–4 months of dialysis.

### SUMMARY

Extended hemodialysis three weekly for 6 hour periods in an 18 month old infant with end stage renal disease reversed the clinical manifestations of uremia. A single layer of a standard Kol dialyzer was used. Cannulation of the superficial femoral artery and sphenous vein with no 14 teflon tips permitted flow rates through the dialyzer of greater than 100 ml/min without the aid of a blood pump. Ultrafiltration during dialysis permitted fluid removal and alleviated hypertension. Cannulation of large vessels obviated repeated clotting

isodes. The infant grew 3 inches and gained pounds during the 6 months of dialysis. The clinical response to dialysis was excellent.

**Conclusion:** On 26/3/69 the infant received a cadaveric renal homotransplant. At the present time (15/3/69) the child is doing well and renal function adequate.

## REFERENCES

- Cherick C F & de Plessier Bowes A. Food values of portous commonly used. J B Lippincott Company Philadelphia 1966 10th edition.
- Fine R N, Korach B M, Grunbaum C M & Lieberman P. Hemodialysis in children. Submitted for publication 1969.
- Franklin S S, Gordon A, Neeman C R & Maxwell M H. Use of a balanced low protein diet in chronic renal failure. *JAMA* 202 477 1967.
- Holliday M. Personal communication.
- Mallick R J, Zakheim S, Coe F L & Mitroneff J. Hemodialysis in children: technique, kinetic aspects related to varying body size and applica-

- tion to salicylate intoxication, acute renal failure and some other disorders. *Medicine* 45 1 1966.
- 6 Lee H A & Sharpstone W. Hemodialysis in pediatrics. *Acta Paediatr Scand* 55 529 1966.
- 7 Moorhead J F, Edwards E C & Goldsmith, H J. Haemodialysis of three children and one infant with a haemolytic uraemic syndrome. *Lancet* i 570 1965.
- 8 Quimston W, Dillard D & Scribner B H. Can pulsation of blood vessels for prolonged hemodialysis. *Trans Amer Soc Artif Intern Organs* 6 104 1960.
- 9 Sprau, N, Fabry T J Jr, Thompson D D & Rubin A L. The use of extracorporeal hemodialysis in the treatment of salicylate intoxication in a 2 year-old child. *Pediatrics* 24 540 1959.
- 10 Williams A V, Hargest T S & Wohltmann H J. Chronic hemodialysis of a 2 year-old child. *Pediatrics* 43 116 1969.

Submitted July 28 1969

Accepted Oct. 9 1969

(C M G.) Childrens Hosp of Los Angeles  
4650 Sunset Boulevard  
Los Angeles  
California 90034  
U.S.A.

**Key words:** Haemodialysis, infant.

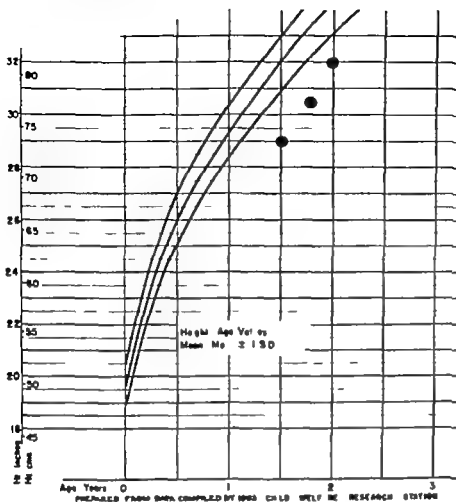


Fig 3 Growth chart

Clotting in the arteriovenous cannula occurred on 4 occasions. The clot was easily removed on the first 2 occasions. The third necessitated surgical revision with a new teflon tip placed in the artery and the fourth necessitated transfer of the cannula to the other leg. The initial cannula functioned for 5 1/2 months. Blood transfusion to raise the hematocrit to 30% was necessary to prepare the patient for both surgical procedures. Clotting of the dialyzer occurred once and a transfusion was needed for replacement.

During three dialyses the systolic blood pressure fell rapidly to 60–70 mm Hg due to excessive ultrafiltration and fluid removal. Hypotension was easily and rapidly corrected by the administration of 0.9 N saline via the venous drip chamber.

**Clinical response.** On admission to the hospital the infant was hypotonic, did not sit with

out support or smile. After 2 months of dialysis she sat without support and after 4 months walked with support. Her affect improved and she began to smile and talk after 3–4 months of dialysis.

## SUMMARY

Extended hemodialysis thrice weekly for 6 hour periods in an 18 month old infant with end stage renal disease reversed the clinical manifestations of uremia. A single layer of a standard Kol dialyzer was used. Cannulation of the superficial femoral artery and saphenous vein with no. 14 teflon tips permitted flow rates through the dialyzer of greater than 100 ml/min without the aid of a blood pump. Ultrafiltration during dialysis permitted fluid removal and alleviated hypertension. Cannulation of large vessels obviated repeated clotting.

the disease and 3 infants died all of them suffering from serious complicating diseases

F Holstad *Staphylococcus aureus* infections in the newborn

J Ek *Staphylococcus aureus* osteomyelitis in the neonatal period

During the period 1956-67 altogether 111 cases of acute hematogenous osteomyelitis were seen in these paediatric wards. The disease occurred relatively more frequently in the neonatal period than in any later age period as many as 16 acquired the disease in the first month of life and in 13 of these cases the etiological agent was proved to be *staphylococcus aureus*. An accumulation was found in the period 1958-62 when *staphylococcus aureus* infections were a serious problem in maternity hospitals.

Multiple bone affections were seen in 4 out of the 13 neonates with *staphylococcus aureus* infection. Three had maxillitis. Local swelling and leukocyte reaction were the most constant positive findings on admission; most patients had normal temperature and ESR and only moderately affected general condition. Five patients had pathological X-ray findings on admission, the others developed this later in the course or the diagnosis was made from material from the infectious focus. Blood cultures gave little diagnostic information in the material.

The prognosis was more serious in the neonatal period compared with infancy or early childhood. 1 died, 3 developed sequelae and 1 of these chronic osteomyelitis.

T Miltvedt *Toxoplasmosis*

A. Wold Høy *Toxoplasmosis* case report

J Marstrand *Loxosoma* two cases

T M Tverdal & E A Nylund *Inclusion Body Disease*

A newborn infant with *Inclusion Body Disease* was described. The infant was asphyxiated at

birth and remained cyanotic with respiratory distress throughout the course.

Icterus developed at the 2nd day of life. There was no hepatosplenomegaly. Eye grounds were normal. The infant was treated for hypoglycemia, convulsions and sepsis. He maternized and heart failure developed at 33 days of age when the infant died. Three examinations for cytomegalic inclusion bodies in urine were negative. Postmortal examination showed inclusion bodies in kidneys, thyroid, pancreas and lungs.

G Fluge, V Lehmann & P J Moe *Hereditary fructose intolerance*

R Lundemann *Tyrosine metabolism in fructosemia*

The acute form of fructosemia in early infancy may show the same clinical picture and the laboratory findings as acute tyrosinosis. Both types of patients may have hepatomegaly, edema, ascites, pathological liver function tests, proteinuria, mellituria, generalized aminoaciduria and striking excretion of phenolic acids.

The levels of the serum amino acids are elevated both in fructosemia and "acute tyrosinosis" but in fructosemia there appears to be a more generalized aminoaciduria. Methionine is very high in both disorders in the acute stage and tyrosine is elevated particularly in acute tyrosinosis. A marked generalized aminoaciduria is found in both disorders. The excretion of proline was particularly high in fructosemia and only moderately increased in "acute tyrosinosis". A marked excretion of the phenolic acids is seen in both types of patients. In the cases with fructosemia the tyrosyluria disappeared as soon as fructose was removed from the diet. It may be concluded that it is not possible to make a differential diagnosis between fructosemia and "acute tyrosinosis" on the basis of the urine and serum amino acids and the phenolic acid excretion when the child is on a normal diet.

## PROCEEDINGS OF PAEDIATRIC SOCIETIES

### NORWEGIAN PAEDIATRIC SOCIETY

Meeting June 12-14 1969

#### *Infections in the neonatal period*

O J Mellbye *Immunological conditions in the newborn*

O Garborg *Neonatal purulent meningitis*

Summary 10 cases of neonatal purulent meningitis were treated at the Children's department Sentralsykehuset, Trondheim from April 1 1963 to April 1 1969. Infants with obvious congenital defects of the central nervous system were excluded.

Three cases were due to *E. coli* 1 to *Aerobacter aerogenes* 1 to *Proteus mirabilis* 1 to *Staphylococcus aureus* 2 to Group B Streptococci and 1 to *Listeria monocytogenes*. In one case no organism could be cultured from the cerebrospinal fluid.

The characteristic features of meningitis in the newborn period were discussed. The importance of Group B streptococci, the high vaginal carrier rate among pregnant women and the cross infections seen in maternity units were emphasized.

The prognosis is poor even with modern therapy. The mortality rate is 50-60% with a high frequency of sequelae in the survivors. In the present series 2 infants died, 3 developed hydrocephalus. At a follow up 1 to 5 years after the acute meningitis 2 patients were apparently without sequelae, 3 were moderately and 3 severely handicapped (mental retardation, convulsions, spasticity, deafness, squint).

Recent studies suggest that maternal carriers of certain bacteria (Group B Streptococci

*Listeria monocytogenes*, *Vibrio fetus*) may comprise a high risk group. The eradication of these organisms from the genital tract during pregnancy may be of great prophylactic value.

J Marstrand *E. coli meningitis*

K B Cyvin *Neonatal pneumonia*

In the period April 63-Dec 68 437 cases of pneumonia were registered at the Department of Pediatrics Sentralsykehuset Trondheim. 39 occurred during the neonatal period, 24 boys and 15 girls. The initial symptoms were in half of the patients noted during the first two days of life. The most common symptoms were cyanosis (13), nasal congestion (12), coughing (9) and apnoeic spells (4). Four infants were febrile, 17 patients were premature. Abnormal pregnancy was noted in 11 cases and pathological delivery was registered in 15 mothers. Relatively often the infants were asphyxiated after delivery. The blood picture was uncharacteristic and bacteriological cultures from nose and throat were of little value for the therapy. In 4 patients the chest X-ray revealed distinct pulmonary infiltrates, whereas the changes were uncertain in 27 cases. Nine infants were treated with penicillin and streptomycin, 8 with ampicillin and 11 with cephaloridine. The remaining group was treated with other combinations of antibiotics. One patient with staphylococcal pneumonia received methicillin and fusidic acid. 26 patients recovered in a few days, 10 showed a protracted course of

in disease and 3 infants died all of them suffering from serious complicating diseases

F Holstad *Staphylococcus aureus* infections in the newborn

J Ek *Staphylococcus aureus* osteomyelitis in the neonatal period

During the period 1956-67 altogether 111 cases of acute hematogenous osteomyelitis were seen in these paediatric wards. The disease occurred relatively more frequently in the neonatal period than in any later age period as many as 16 acquired the disease in the first month of life and in 13 of these cases the etiological agent was proved to be staphylococcus aureus. An accumulation was found in the period 1958-62 when staphylococcus aureus infections were a serious problem in maternity hospitals.

Multiple bone affections were seen in 4 out of the 13 neonates with staphylococcus aureus infection. Three had maxillitis. Local swelling and leukocyte reaction were the most constant positive findings on admission most patients had normal temperature and ESR and only moderately affected general condition. Five patients had pathological X ray findings on admission the others developed this later in the course or the diagnosis was made from material from the infectious focus. Blood cultures gave little diagnostic information in the material.

The prognosis was more serious in the neonatal period compared with infancy or early childhood. 1 died 3 developed sequelae and 1 of these chronic osteomyelitis.

T Mjølvedt *Toxoplasmosis*

A Rold Haug *Toxoplasmosis* case report

J Marstrand *Lues congenita* two cases

T M Tverdal & E A Mylius *Inclusion Body Disease*

A newborn infant with Inclusion Body Disease was described. The infant was asphyxiated at

birth and remained cyanotic with respiratory distress throughout the course.

Icterus developed at the 2nd day of life. There was no hepatosplenomegaly. Eye grounds were normal. The infant was treated for hypoglycemia, convulsions and sepsis. Hematemesis and heart failure developed at 33 days of age when the infant died. Three examinations for cytomegalic inclusion bodies in urine were negative. Postmortem examination showed inclusion bodies in kidneys, thyroid, pancreas and lungs.

G Fluge, V Lehmann & P J Moe *Hereditary fructose intolerance*

R Lindemann *Tyrosine metabolism in fructosemia*

The acute form of fructosemia in early infancy may show the same clinical picture and the laboratory findings as "acute tyrosinosis". Both types of patients may have hepatomegaly, edema, acute pathological liver function tests, prothrombin, mellituria, generalized aminoaciduria and striking excretion of phenolic acids.

The levels of the serum amino acids are elevated both in fructosemia and "acute tyrosinosis" but in fructosemia there appears to be a more generalized aminoaciduria. Methionine is very high in both disorders in the acute stage and tyrosine is elevated particularly in acute tyrosinosis. A marked generalized aminoaciduria is found in both disorders. The excretion of proline was particularly high in fructosemia and only moderately increased in "acute tyrosinosis". A marked excretion of the phenolic acids is seen in both types of patients. In the cases with fructosemia the tyrosyluria disappeared as soon as fructose was removed from the diet. It may be concluded that it is not possible to make a differential diagnosis between fructosemia and "acute tyrosinosis" on the basis of the urine and serum amino acids and the phenolic acid excretion when the child is on a normal diet.



## PROCEEDINGS OF PAEDIATRIC SOCIETIES

### NORWEGIAN PAEDIATRIC SOCIETY

Meeting June 12-14, 1969

#### *Infections in the neonatal period*

O J Mellbye *Immunological conditions in the newborn*

O Garborg *Neonatal purulent meningitis*

Summary 10 cases of neonatal purulent meningitis were treated at the Children's department Sentralsykehuset i Trondheim from April 1 1963 to April 1 1969 Infants with obvious congenital defects of the central nervous system were excluded

Three cases were due to *E. coli* 1 to *Aerobacter aerogenes* 1 to *Proteus mirabilis* 1 to *Staphylococcus aureus* 2 to Group B Streptococci and 1 to *Listeria monocytogenes* In one case no organism could be cultured from the cerebrospinal fluid

The characteristic features of meningitis in the newborn period were discussed The importance of Group B streptococci the high vaginal carrier rate among pregnant women and the cross infections seen in maternity units were emphasized

The prognosis is poor even with modern therapy The mortality rate is 50-60% with a high frequency of sequelae in the survivors In the present series 2 infants died 3 developed hydrocephalus At a follow up 1 to 5 years after the acute meningitis 2 patients were apparently without sequelae 3 were moderately and 3 severely handicapped (mental retardation, convulsions spasticity deafness squint)

Recent studies suggest that maternal carriers of certain bacteria (Group B Streptococci

*Listeria monocytogenes Vibrio fetus*) may comprise a high risk group The eradication of these organisms from the genital tract during pregnancy may be of great prophylactic value

J Marstrand *E. coli meningitis*

K B Cyvin *Neonatal pneumonia*

In the period April 63-Dec 68 437 cases of pneumonia were registered at the Department of Pediatrics Sentralsykehuset Trondheim occurred during the neonatal period 24 boys and 15 girls The initial symptoms were in 16 of the patients noted during the first two days of life The most common symptoms were cyanosis (13) nasal congestion (12) coughing (11) and apnoeic spells (4) Four infants were febrile 17 patients were premature Abnormal pregnancy was noted in 11 cases and pathological delivery was registered in 15 mothers Relatively often the infants were asphyxiated after delivery The blood picture was uncharacteristic and bacteriological cultures from nose and throat were of little value for therapy In 4 patients the chest X-ray revealed distinct pulmonary infiltrates whereas the changes were uncertain in 27 cases Nine infants were treated with penicillin and streptomycin 8 with ampicillin and 11 with cephaloridine The remaining group was treated with other combinations of antibiotics One patient with staphylococcal pneumonia received methicillin and fusidic acid 26 patients recovered in a few days 10 showed a protracted course of

Table 1 *Australia antigen in blood diseases and malignancy*

	No tested		Au positive		Antigen? No
	Children	Adults	Children	Adults	
<b>Leukemia</b>					
Acute stem cell	35	0	3	0	(1)
Acute myeloblastic	5	0	1	0	
Chronic lymphocytic	0	3	0	1	
Other types	1	1	0	0	
Hodgkin's Disease	3	6	1	1	(1)
Lymphosarcoma	2	0	0	0	
Reticulosarcoma	7	0	0	0	
Solid tumor	9	0	0	0	
Osteop. tumor	2	0	0	0	
Various other blood dis.	20	0	0	0	(1)
<b>Total</b>	<b>84</b>	<b>16</b>	<b>5</b>	<b>2</b>	<b>(3)</b>

rem from a child with acute leukemia. This child had not received any blood transfusion or hepatotoxic drugs.

A new precipitating antigen-antibody system (Pennsylvania) has recently been discovered. Pennsylvania antigen seems to occur in about the same diseases as Au antigen.

#### *J. Steen. Syndrome Kallmann sive de Morsier*

This syndrome is rare. Mostly boys are affected and they have pituitary hypogonadism and anosmia.

The patient demonstrated is born in 1949. He has been under observation the last 5 years due to deficient pubertal development. No known relatives with the same syndrome. The patient has never had any serious diseases or injuries. Normal psychic development. Slow growth of height from the age of eleven. He is now 168 cm tall. Approximately normal proportions of the body. No gynecomastia. Infantile testis about 1-2 cm both descended.

Apart from scarce pubic hair, no secondary sexual characteristics. Normal PBI, normal ACTH secretion both by Metopirontest and in direct serum tests. Very low or lacking gonadotropin in repeated tests. Testosterone in plasma subnormal. Sex chromatin negative. He has a total anosmia.

The only possible differential diagnosis is

pubertas tarda. The patient has for the last year been treated with human gonadotropin in cooperation with Pediatric Research Institute Rikshospitalet O lo. Despite the fact that there has been an increase of testosterone in plasma up to lower normal values, there can still not be observed any clinical effect of the treatment.

#### *k. H. Torp. Menarche in North Norway*

*J. Steen. Johnsen. Coma subsequent to measles vaccination*

Encephalitis is reported to occur in 1-4% of measles patients. More than 50% of children with uncomplicated measles present abnormal EEG. The last years the central nervous disease SSPE (subacute sclerosing panencephalitis) has been related to measles. Years following the acute disease the cerebral degeneration occurs presenting with increased measles antibody and living virus in the nervous tissue.

Living measles virus vaccine has recently been suspected to cause encephalitis in England (Beckenham 20 strain). A more attenuated strain is used in Norway (Schwarz). From U.S.A. 23 possible encephalitis cases were reported in 14.8 million inoculations with living vaccine, a rate far below the spontaneous measles encephalitis.

The possibility of measles vaccine virus

It is therefore of importance to exclude fructosæmia in cases presenting the clinical picture of acute tyrosinosis in infancy

#### H Pande & E Ruud *Lowe's syndrome*

Lowe's syndrome is a hereditary disease in the group inborn errors of metabolism associated with aminoaciduria first described in 1952. The basic biochemical defect is unknown. It is characterized by the following main symptoms: bilateral congenital cataract and/or glaucoma, psychomotor retardation, retarded growth, generalized hyperiminoaciduria, proteinuria, metabolic acidosis, rickets, hypotonia and in some cases glycosuria. The affected child has a characteristic look. Two brothers suffering from Lowe's syndrome were reported. They both had findings typical of the disease. The elder one had rickets which responded well to 460 000 units of vitamin D for a course of nine days. A brief discussion of the different symptoms were given.

#### A B Cyvin *Two siblings with Lowe's syndrome*

Two siblings, a boy and a girl with the characteristic features of the oculo-cerebro-renal syndrome of Lowe are described. In both cases the impaired renal function was a predominant feature of the disease and both died at the age of 3-4 months.

#### J Weidemann *Ophthalmological findings in Lowe's syndrome*

#### T Feyling *Subcutaneous fat necrosis with hypercalcaemia in infants*

A case of neonatal subcutaneous fat necrosis with evidence of hypercalcaemia was described. The eight cases from the literature were briefly discussed.

The serum calcium levels decreased to normal after administration of prednisone and limitation of dietary vitamin D and calcium.

No complications are apparent after 10 months.

#### *Malignant diseases in children*

#### E Bjerke *The frequency of malignant diseases in children in Norway*

#### O Knutrud *Diagnosis and treatment of malignant tumors*

#### P J Moe *Treatment of malignant diseases with cytostatics*

#### M Seip *Treatment of acute leukemia*

#### P J Moe *Antigen-antibodies in malignant blood diseases*

The Australia precipitating antigen antibody system was discovered by Blumberg *et al.* in 1964. Australia (Au) antigen is demonstrated by a double diffusion technique using a micro-Ouchterlony technique. The antigen may also be seen with an electron microscope after ultracentrifugation on a sucrose gradient. It is about the same size as has been postulated for hepatitis virus and it is possible that Au antigen is the same type of virus.

Au antigen occurs extremely rarely in American and Scandinavian populations. Blumberg and co-workers have recently shown that Au antigen occurs in about 10-15% of cases of acute myelogenous leukemia, chronic lymphatic leukemia, acute lymphatic leukemia and Hodgkin's disease and in about 30% of institutionalized patients with Down's syndrome. Au antigen also seems to be a temporary finding in serum from about 20% of patients with hepatitis.

A preliminary report is given of the results of Au antigen studies performed during the last year on serum from Norwegian children (and a few adults). The serum studies were performed by Dr L. Melartin, Helsinki. The incidence of Au antigen in leukemia and Hodgkin's disease in childhood (Table 1) was found to be about the same as stated above. Negative reactions were found in sera from 40 children with other malignant diseases or other blood disorders except for a questionable positive test (positive reaction to human antiserum and negative to rabbit antiserum) in a child with congenital hypoplastic thrombocytopenia. Anti Au was demonstrated in a

# NEW BOOKS RECEIVED

- L. P. Hollander & M. Matthys (eds) *Ergebnisse der Elektroenzephalographie* Vol X Bibliotheca Haemorrhologica No 32 348 pp illus S Karger AG Basel New York 1969 sFr 79 US \$18.95
- M. Lloyd Rustby (ed) *New ideas in asthma and its management* 56 pp The Chest and Heart Association Health Horizon Ltd London 1969 10s
- J. Vialatte *Les enfants allergiques* 164 pp Expansion Scientifique Française Paris 1969 32 F
- E. Jom (ed) *Endokrinologische Probleme im Kindesalter* Pädiatrische Fortbildungskurse für die Praxis Vol 25 190 pp illus S Karger AG Basel New York 1969 DM 39
- O. Koldovsky *Development of the functions of the small intestine in mammals and man* 204 pp illus S Karger AG Basel New York 1969 DM 53
- A. Adamsons (ed) *Diagnosis and treatment of fetal disorders* 304 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 59.20
- T. Bannister & R. Frey (eds) *Epilepsie im Kindesalter* Pädiatrische Fortbildungskurse für die Praxis Vol 26 136 pp illus S Karger AG Basel New York 1969 DM 33
- D. Hubble (ed) *Pediatric Endocrinology* 492 pp illus Blackwell Scientific Publ Oxford 1969 67 10s
- J. L. Miesack (ed) *Progress in Neonatal Neurology* Vol 11 449 pp illus S Karger AG Basel New York 1969 DM 89 US \$21.35
- U. Brunner *Das Lymphödem der äußeren Extremitäten* 248 pp illus Verlag Hans Huber Bern 1969 DM 44
- P. G. Jure & L. P. Lieberman *Personality disorders in adolescence* 398 pp illus Akademika Kádó Budapest 1969 US \$24.00
- O. H. W. Wohstenholme & H. O. Connor (eds) *Foetal autonomy* A Ciba Foundation Symposium 326 pp illus J & A Churchill Ltd London 1969 70s
- L. Moore *Lezigue Française Anglais Allemand des termes médicaux en pédiatrie neuro-psychiatrie infantile et psychologie pathologique* 224 pp Expansion Scientifique Française Paris 1969 24 F
- P. Gardner R. Mac Keith & V. South (eds) *Aspects of developmental and paediatric ophthalmology* Clinica in Developmental Medicine 32 126 pp illus W Heinemann Medical Books Ltd London 1969 42s
- L. Duthelm O. Olsson F. Strand R. Vreten & A. Zetterqvist (eds) *Handbuch der medizinischen Radiologie* Vol X Teil 1 *Röntgenradiagnostik des Herzens und der Gefäße* (H. Vreten (ed)) 789 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 296 US \$74.00
- H. Öberg *Harnwegsinfektion und Harnbefund bei Kindern* 112 pp illus Georg Thieme Verlag Stuttgart 1969 DM 34
- G. V. Power (ed) *Mental retardation. Diagnosis and treatment* 284 pp illus Hoeber Medical Division Harper & Row Publ New York 1969 US \$9.50
- S. B. Zerkow *Technic of roentgenologic investigation* 318 pp illus Akademika Kádó Budapest 1969 US \$4.00
- H. Heyck & G. Lenzahn (eds) *Die progressiv-dystrophischen Myopathien* 436 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 118
- P. L. Lichten *Ähnliche Vektor Elektrokardiographie* 235 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 86

causing SSPE is suspected but not proved. A 2 year old girl was admitted to the Childrens Department Porsgrunn one week after measles vaccination. She was in coma with clinical and EEG signs of encephalitis. A strong curry-like smell was later on recognized. She had since 9 months of age suffered some episodes with febrile convulsions. Diagnostic virus investigations revealed no growth of measles virus and the antibody response was very weak. Aminoacid chromatography in urine and blood showed great excess of leucin, isoleucine and valin. Following 10 days treatment with low protein diet the girl was in clinical and

biochemical normal condition. She is not mentally retarded.

Seven children is previously known with the late manifesting variant of Maple Syrup Urine Disease, 3 of these from Norway (dr R. Kjel). The measles vaccination with viremia has probably precipitated the further derangement in this inborn enzymatic error of metabolism and no real encephalitis was proved.

Children with previous convulsions should not be given measles vaccine.

O. Garborg *Nutrition problems in East Africa*

*Thor Østern Endre*

NEW BOOKS RECEIVED

- L. P. Hollander & M. Matthies (eds) *Ergebnisse der Blutraufnahmeforschung* Vol. X Bibliotheca Haematologica No 32 348 pp illus 5 Karger AG Basel New York 1969 sFr 79 US \$18.95
- N. Lloyd Rusbly (ed) *New ideas in asthma and its management* 56 pp The Chest and Heart Association Health Horizon Ltd London 1969 10s
- J. Vialatte *Lesions allergiques* 164 pp Expansion Scientifique Française Paris 1969 32 F
- E. Joss (ed) *Endokrinologische Probleme im Kindesalter Pädiatrische Fortbildungskurse für das Praxis* Vol. 25 190 pp illus 5 Karger AG, Basel New York 1969 DM 39
- O. Koldovsky *Development of the functions of the small intestine in mammals and man* 204 pp illus 5 Karger AG Basel New York 1969 DM 53
- K. Adkinson (ed) *Diagnosis and treatment of food disorders* 104 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 59.20
- Th. Bateman & R. Fry (eds) *Epilepsy im Kindesalter Pädiatrische Fortbildungskurse für das Praxis* Vol. 26 136 pp illus 5 Karger AG Basel New York 1969 DM 33
- H. Hobbie (ed) *Pediatric Endocrinology* 492 pp illus Blackwell Scientific Publ Oxford 1969 £7 10s
- J. L. Meirick (ed) *Progress in Medical Virology* Vol. 11 461 pp illus 5 Karger AG Basel New York 1969 DM 89 US \$21.35
- U. Bruchner *Das Lymphödem der unteren Extremitäten* 48 pp illus Verlag Hans Huber Bern 1969 DM 44
- P. O. Joss & L. P. Liebermann *Pernichlektie im Augen im Kindesalter* 598 pp illus Akademik Kádó Budapest 1969 US \$24.00
- Q. E. W. Wolstenholme & M. O. Connor (eds) *Foetal autonomy* A Ciba Foundation Symposium 326 pp illus J & A Churchill Ltd London 1969 70s
- L. Moore *Langue Française Aspects Aléatoires des termes usés en psychiatrie neuro-psychiatrie en pédiatrie et psychologie pathologique* 274 pp Expansion Scientifique Française Paris 1969 24 F
- F. Gardner R. Mac Keith & V. Smith (eds) *Aspects of developmental and paediatric ophthalmology Clinica in Developmental Medicine* 32 126 pp illus W. Heinemann Medical Books Ltd London 1969 42s
- L. Duthelm O. Olsson F. Strand H. Vieten & A. Zippinger (eds) *Handbuch der medizinischen Radiologie* Vol. X Teil 1: *Röntgendiagnostik des Herzens und der Gefässe* (H. Vieten (ed)) 789 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 296 US \$74.00
- H. Olberg *Harnwegsinfektion und Harnröhrenfunktionsstörungen* 112 pp illus Georg Thieme Verlag Stuttgart 1969 DM 34
- Ch. M. Power (ed) *Mental retardation Diagnosis and treatment* 284 pp illus Hoeber Medical Division Harper & Row Publ New York 1969 US \$9.50
- S. B. Zentgraf *Technique of roentgenologic investigations* 318 pp illus Akademik Kádó Budapest 1969 US \$24.00
- H. Heyck & O. Lindahn (eds) *Die progressiv-dystrophischen Myopathien* 436 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 118
- P. Lichtlen *Klinische Vektor-Elektrokardiographie* 235 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 86

# BOOK REVIEWS

Charles Varga *Handbook of pediatric medical emergencies* The C V Mosby Co St Louis Missouri 1968 694 pp \$19.75

The success of the future generation lies dormant in this child. Therefore in this hour the future of tomorrow lies in your hands. How then will you shape his destiny and that of the world? Yours is an awesome responsibility. Fulfill that responsibility as if your very life depended upon it.

The demand expressed in this quotation from J C Edwards one of the 14 contributors to the book is really an urgent one. There is great need for good advice in medical emergencies. The 4th edition of this handbook discusses almost all pediatric medical emergencies including cardiovascular neurologic psychiatric respiratory endocrine hematologic ophthalmologic genitourinary and metabolic emergencies. There are also articles on emergencies in the newborn on emergencies due to physical and chemical agents and due to hypersensitivities and toxins. It is not a task for a general paediatrician to value the facts given by different experts but as far as I can find these facts are up-to-date and in accordance with routine treatment at a pediatric clinic. A little too routine perhaps. The greatest advantage of this handbook is the surveyable arrangement of the text. One can rapidly find the facts elucidating the acute situation. Therapeutic doses of drugs are arranged in tables. The doses are correlated to body weight or surface area throughout the book and there are many short useful notes and comments. About 90 pages of the book deal with the problems of poisoning. Here I miss information on toxic doses. In Sweden we have had experiences of the good effect of copper sulphate as an emetic. In this book copper sulphate is mentioned only as a poison. One of the most informing chapters deals with pediatric procedures. There are many illustrations and most of them are very good. There is however one illustration which in my opinion is somewhat misleading showing superficial temporal veins suitable for scalp vein puncture. My experience is that those vessels are very fragile and inferior to those on the forehead. There are about 1000 references. This handbook of pediatric medical emergencies can be recommended to be read by anyone who may get in such a situation and ought to be available for rapid consultation in every emergency room. Thereby it can surely make the doctor's awesome responsibility a little bit less awesome.

Bo Ballard

R Mac Keith & M Bax (eds) *Studies in infantile Clinics in Developmental Medicine* No 27 W Hensmann Medical Books Ltd London 1968 109 pp 25s

This booklet is mainly based on work dealing especially with neonatal neurology presented at the meeting of the 5th International Study Group at Oxford in 1966.

In 40 pages Prechtl and his coworkers describe their extensive polygraphic studies on full term newborns. The standard variables recorded were respiration EKG EEG EMG and eye movements. Some papers are dealing with abnormal neurological conditions e.g. convulsions from endocrinological point of view and characteristics of fits in the newborn period. The relationship between perinatal incidence and later development of the children is discussed in five papers. In the reviewer's opinion this is the most valuable part of the book emphasizing not only the value of detailed studies on a newborn infant but also the difficulty in drawing prenosological conclusions from the findings of these studies.

The value of such a book is that the reader gets in a condensed form the experiences from various groups in the world on some special problems.

Tor Lindberg

J Brodehl *Der renale Transport der Aminosäuren im Säugling und Kindesalter* Ferdinand Enke Verlag Stuttgart 1969 97 pp DM 30.—

Unter Verwendung von automatisierter Säulenchromatographie bestimmte der Verf. die freien Aminosäuren im Serum und Urin sowie den Insulin und PAH Clearance unter Standardbedingungen bei Säuglingen und Kindern. Der Verf. gibt seine Resultate in dieser sehr sorgfältig durchgeführten Untersuchung über den renalen Aminosäuretransport wieder. Er diskutiert seine Resultate mit den bisher nur im geringen Umfang vorliegenden Resultaten anderer Untersuchungen und schließt damit eine wichtige Lücke. Die Resultate des Verf. können somit als gute Referenzbasis für andere Untersuchungen bei ihren klinischen Studien über Aminosäuretransport Problemen dienen.

J. Studrud

## THE SIGNIFICANCE OF THE DEFICIENCY STATE IN LESCH-NYHAN DISEASE

H GHADIMI, C K BHALLA and D M MARCHENBAUM

From the Department of Pediatrics, Methodist Hospital of Brooklyn and the  
Department of Biochemistry, State University of New York, New York, U.S.A.

X-linked hyperuricemia associated with destructive finger and lip biting, choreoathetoid movements, spasticity and mental retardation was first recognized as an entity distinct and different from adult gout by Leach & Nyhan in 1964 (5). Since the most obvious and widely recognized biochemical finding is increased concentration of uric acid in the blood and uric conventional therapy in all twenty-two cases so far described has been aimed at reduction of the uric acid level in blood.

Perhaps by analogy with adult gout reduction of blood uric acid has been attempted by institution of a low purine, low protein diet. Contrary to this opinion we believe that institution of a high protein diet and provision of purine precursors is essential to the treatment of these patients.

### TECHNIQUE

Blood samples for uric acid analysis were taken in dry heparinized tubes and centrifuged to obtain the plasma. This was then deproteinized by adding 1 ml of 10% trichloroacetic acid (1:4 V/V). The mixture was centrifuged and the supernatant removed. Assuming that packed cell precipitate contains the same concentration of free uric acid as the supernatant fraction, 5 ml of supernatant represent an aliquot of 1 ml of plasma. Twenty-four hour urine samples were collected in

bottles containing a small crystal of thymol and kept refrigerated. The volume was measured and a small amount filtered. A 5 ml aliquot of filtered urine was deproteinized by the addition of 1% picric acid (1:4 V/V).

An aliquot of the picrate supernatant representing 1 ml of plasma or urine was pipetted into a column of Dowex 2X8 (ionic form Cl<sup>-</sup>) to remove the picrate. The column was washed six times with 2 ml portions of 0.01 N HCl and the combined effluent evaporated to dryness on a rotary evaporator. The temperature of the water bath did not exceed 40°C. The dry samples prepared in this manner were stored in the deep freeze. Since it has been our experience that the glutamine in samples is variable even in the dry state in the deep freeze, blood samples were analyzed by column chromatography on the day they were drawn. Two cerebrospinal fluid samples were obtained and treated in the same way.

Free amino acids were measured by the use of an amino acid analyzer built according to the Pitts modification (11) of the technique originally described by Spackman *et al.* (14). In our previous studies on cerebrospinal fluid it became mandatory to separate glutamine from adjacent peaks. This was eventually achieved by adjustment of the column temperature. Two tenths mmole of norleucine was added to the samples before application to the column as an external standard for checking the color yield. Judged by the recovery of this substance, the reproducibility is  $\pm 4\%$ .

Uric acid was determined by the use of an automatic analyzer based on the method described by Newton (7). Three tenths ml of diluted urine (1:10) and 0.3 ml of serum were applied to the analyzer. Based on application of 10 mg uric acid as an internal standard, the reproducibility of the technique is  $\pm 3.0\%$ .

The method used for hypoxanthine guanine phosphoribosyl transferase (HGPRTase) activity determination by Dr Nyhan's laboratory is a modification of that described by Kornberg *et al.* (4).

This study was supported by Research Contract 1 RO1 HD 0379-01 from the National Institute of Child Health and Human Development.





Fig 1 Patient prior to treatment. Note spasticity and strong urge to bite fingers

## CASE REPORT

Patient A S was the product of an uneventful full term pregnancy, low forceps delivery weighing five pounds twelve ounces at birth. The parents were not related. The maternal great grandmother and great great grandfather had cleft A male first cousin of the maternal grandmother died at eight months because of continuous illness. Otherwise the clinical family history is non-contributory. The patient's neonatal period was reported uneventful except that each feeding took a long time. No developmental abnormality was noticed by the parents until the baby was six months old. It was then observed that the infant could not sit even with support.

At one year of age his head circumference was 40.5 cm, his height was 70 cm (below 3rd percentile) and he weighed 9.5 kg (25th percentile). He could roll over, lift his head, lean forward on his hands and reach out to grasp large objects (with some tremors). He attempted to receive dropped objects and responded to his name, played peek-a-boo and repeated some consonant sounds e.g. cat, Ma. He could not sit without support, transfer objects from one hand to another, pull himself to a standing position or grasp objects with his thumb and forefinger. He cried when he was hungry.

On physical examination at one year of age except for hypotonia, physical retardation and small stature, no other abnormality was noticed. Reflexes were within normal limits. His plasma and urine free

amino acids examined by column chromatography were within the normal range. Blood uric acid was determined although the patient had no history of finger biting or spasticity. The value was 52 mg/100 ml. The parents were advised to bring the child after six months for re-evaluation.

The patient returned at 3 1/2 years of age revealing a history of finger biting which started at two years, spasticity and increasing irritability. His blood uric acid at this time was 8.8 mg/100 ml.

The patient was admitted with the diagnosis of Lesch-Nyhan Disease. Scrutiny of the dietary history revealed that his diet generally consisted of the following: *Breakfast*—1/2 to 1 slice of toast, 1 cup or less of cereal and 1/2 liter of juice. *Lunch*—1 cup of squash or spinach, 1/2 cup macaroni, one piece of cake for dessert and 1/2 liter juice. *Snack*—several cookies. *Dinner*—1/2 cup vegetables, potato, 10-15 g meat and 1/2 liter juice. Based on the above items, the daily intake was less than 1000 Calories and less than 10 g protein per day.

Physical examination on admission revealed a hyperirritable, poorly nourished and poorly developed child. His weight was 9.9 kg and his height was 76 cm, both below the third percentile. He constantly said 'juice' asking for a drink. He screamed when approached and went into opisthotonus. His face had a vacant look. His arms were splinted and his hands were gloved. On removal of the elbow splints and gloves his fingers immediately went to his mouth. Hard biting and bleeding followed. His action in this respect was similar to an addict craving drugs. Raw wounds from previous bites were observed on all the finger tips. He did not bite his lips (Fig 1).

Neurological consultation records a hopelessly retarded child. His speech was not distinct and he was staring in space. His back and neck were constantly in opisthotonus. He showed marked hyperirritability and spasticity. The child kept his legs in a scissoring position. Deep tendon reflexes were brisk. Plantar responses were extensor. Ankle clonus was sustained. Response to painful stimuli was normal. Examination of other systems revealed no abnormality.

Routine analysis of blood, urine and CSF as well as EEG and skull X-ray did not show any abnormal result. X-ray of the wrist suggested a bone age of approximately 2 years.

## INVESTIGATION

Preliminary investigation during the first two weeks of hospitalization consisted of 1) close clinical observation of the patient's condition with particular emphasis on his diet, 2) repeated analysis of the patient's blood, urine and cerebrospinal fluid for amino acids and uric acid, 3) chromosomal and enzymic studies of the patient's blood. No amount of perna

Table 1 Serum and urinary uric acid values of the patient and his immediate relatives compared to controls

Patients' serum uric acid obtained at one year not included

Subject	Age (years)	Serum uric acid (mg/100 ml)	Urinary uric acid	
			mg/24 hrs	mg/g creatinine
Patient	3½	8.8-15.0	369.0	3719.0
Sister	13	4.3		
Father	28	5.4	148.2	200.0
Mother	26	3.9	704.5	393.3
Maternal grandfather	59	5.2		
Maternal grandmother	47	4.1	993.0	543.4
Maternal uncle (M)	18	5.3	187.0	474.1
Maternal uncle (J)	23	5.8-6.2	521.4	1372.4 <sup>a</sup>
Controls for serum				
12 Children (7 M, 5 F)	3-6	3.6 (2.5-5.9)		
23 Male adults		3-7.1		
1 Female adults		2.6-5.2		
Controls for urine				
11 Children (3 M, 8 F)	5 mos-6 yrs		62.05 (1.8-291.5)	761.0 (175.0-2166.6)
7 Male adults			527.0 (440.0-710.0)	342.0 (74.3-406.0)

Before treatment

Based on determinations

Based on 3 determinations

acid could induce the patient to accept any high protein food including raw or cooked hamburger bloodwurst etc. The results of the blood and urinary uric acid determination of the patient and several of his close relatives are presented in Table 1. Chromosomal studies were normal.

Two weeks after hospitalization allopurinol therapy (150 mg/day) was started. The response of the blood uric acid level was dramatic: within five days it was reduced to 2.5 mg/100 ml.

Excessive formation and excretion of uric acid which requires glutamine, glycine and aspartic acid in the face of prolonged low protein intake indicated to us the necessity for administration of a high protein diet in association with allopurinol therapy. After 14 days of allopurinol therapy the patient still could not be persuaded to take the prescribed amount of protein. Studies of free amino acids of the blood samples taken at one year of age and during the first four weeks of hospitalization showed persistently low glutamine con-

centrations (8.4, 4.4 and 2.5 mg/100 ml—See Table 2). Since monosodium glutamate (MSG) is commercially available for the purpose of making food more appetizing it was added to the diet in the amount of 10 g/day. The patient liked the taste and his appetite steadily improved.

Six weeks after admission that is two weeks following the administration of MSG his daily intake rose to 38-70 g protein per day and 1000-1500 Calories per day. At this time there was a definite change in his disposition as judged by the House Staff members, nurses and neurologist. He became a smiling happy "hello-good-by" child with a "winning person ality" as the neurologist later recorded. His legs were relaxed while in a supine position. While holding him in a standing position he initiated walking. His screaming and stiffness decreased and opisthotonus disappeared. He started to answer simple questions such as "How are you?" and "Where is Mommy?" He could sit in his walker and cruise about using his toes most of the time. The urge to

Table 2 Free amino acid concentrations of the patient's plasma before and after the administration of monosodium glutamate (MSG) as compared to control values

Three samples were obtained prior to MSG therapy 16 samples were obtained while on treatment

	Plasma free amino acids before MSG Mean Range	Plasma free amino acids from 10 child controls Mean Range	Plasma free amino acids after MSG Mean Range
Glutamine	51 2.5-8.4	12.1 5.9-17.1	9.8 6.1-18.6
Glutamic acid	0.9 0.6-1.2	1.2 T-3.3	1.7 0.5-7.1
Glycine	1.1 0.7-1.3	1.1 0.8-1.4	1.7 0.5-2.9
Aspartic acid	0.2 0.1-0.4	0.1 T-0.2	0.2 0-0.5
Threonine	1.1 0.2-1.8	1.4 0.8-1.8	1.9 0.7-3.5
Serine	1.4 0.7-2.2	1.1 0.6-1.7	1.8 0.7-3.9
Proline	2.3 1.6-3.3	1.9 0.8-3.3	2.8 1.4-5.6
Alanine	3.7 1.9-5.5	2.3 1.4-3.8	4.4 1.7-8.5
Cystine	0.8 0.03-2.2	0.5 0-0.8	1.1 0-2.2
Methionine	0.3 0.1-0.6	0.2 0.02-0.4	0.3 0.1-0.7
Leucine	2.0 0.9-4.3	1.6 0.7-2.3	2.2 0.9-4.2
Isoleucine	1.1 0.4-2.4	1.0 0.4-1.1	1.2 0.4-2.4
Tyrosine	1.5 0.4-3.4	0.9 T-1.6	1.7 0.5-3.2
Lysine	2.7 1.7-3.7	3.2 1.2-5.2	2.8 1.4-5.4
Histidine	1.3 1.2-1.3	1.6 1.4-1.8	1.8 0.7-2.8
Phenyl alanine	2.0 0.5-3.4	0.9 T-1.6	1.1 0.2-2.3
Valine	2.9 1.4-5.8	2.5 1.4-3.5	4.0 1.6-8.6

T = Present in trace amount

bite his fingers lessened but the habit persisted when his splints and gloves were completely removed. After a few minutes his fingers found their way to his mouth but were withdrawn immediately by the attendant. On one occasion, he did bite his fingers.

The patient received no special nursing care, physical therapy etc. His mother visited him frequently, but provided no special care or instruction. She was shown how to accurately

determine his daily intake by the use of a dietary balance. The patient left the hospital weighing 11.2 kg; that is, he gained 1.3 kg in the last month of his hospitalization. His blood uric acid was 4.3 mg/100 ml and cerebrospinal fluid uric acid was 0.3 mg/100 ml.

### FOLLOW UP

The patient is attending our clinic every two weeks. His mother brings a daily dietary sheet, which reports the daily intake of protein and calories. Blood and urinary uric acid are measured at each visit; plasma amino acids are determined less frequently.

The patient's dietary intake has remained essentially as at the time of discharge, i.e. protein 38-70 g/day, calories 1000-1500/day, but his weight has not increased at the same rate. At the time of writing the patient is 5½ years old, he weighs 13.9 kg, his height is 96 cm and his head circumference is 45 cm.

The child can roll over. He sits without support and can pull himself to a standing position.



Fig. 2 Patient after 6 weeks of treatment. Note contrast to Fig. 1.



C<sup>14</sup> into uric acid only two or three times as much as normal

The salient biochemical features of Lesch-Nyhan Disease therefore consist of the following: 1) Increased concentration of blood uric acid, resulting in excessive urinary output of that substance; 2) Increased concentration of oxypurines, intermediary metabolites of purine de novo synthesis, in CSF<sup>1</sup> and perhaps in brain; 3) Increased concentration of adenyllic acid in the red blood cells (13); and 4) Absence of the enzyme HG PRTase, which can easily be demonstrated by studies on the red cells (13).

The blood uric acid level of our patient ranged from 8.8 to 15 mg/100 ml before allopurinol therapy. However, the single determination performed at one year of age showed a value of 5.2 mg/100 ml, which is around the upper limit of the conventionally accepted normal range. The lesson to be learned in this respect is that blood uric acid, although the most convenient test, should not be used as the sole criterion for the diagnosis.

We have not measured the oxypurines in the biological fluids of our patient, but enzyme studies on the blood of our patient carried out through the courtesy of Dr Nyhan upheld our diagnosis. The rate of production of IMP from hypoxanthine and GMP from guanine were respectively 0.03% and 0.04% of normal, which are indistinguishable from zero. With the absence of HG PRTase established, the possibility of loss of enzyme during shipment was examined through measurement of the sister enzyme A PRTase. The activity proved to be 177% of normal. This compensatory increase of adenine enzyme is also a classic finding of the disease.

The cardinal neurological manifestation relates to a malfunction in the area of the basal ganglia. Rosenbloom *et al* (12) have recently shown that the basal ganglia of normal adults are particularly rich in HG PRTase. They examined basal ganglia, frontal lobe, cerebellum, spinal cord, ovary, liver, spleen, kidney, muscle, pancreas, jejunum and adrenal tissues

from controls and found that basal ganglia had the highest activity. Basal ganglia of one patient with Lesch-Nyhan Disease tested by the same technique did not show any activity.

The biochemical consequences of HG PRTase deficiency are increased concentrations of uric acid, hypoxanthine, xanthine, and probably adenine, as well as depletion of guanine and possibly of purine precursors from the cells. It is not known which of these secondary biochemical anomalies is responsible for the neurological symptoms or through which mode of action the symptoms are produced.

The most conveniently recognized biochemical abnormality is hyperuricemia, which can be expediently controlled by uricosuric agents. Allopurinol, which inhibits xanthine oxidase, reduces blood uric acid at the expense of increased concentrations of xanthine and hypoxanthine. It is doubtful that hyperuricemia per se plays any role in the production of symptoms other than thirst. After all, none of the neurological manifestations of the disease have been observed in adult gout.

Our most recent observations indicate that periodic withdrawal of allopurinol therapy with the concomitant hyperuricemia does not change the patient's disposition nor cause recurrence of scissoring, spasticity, opisthotonus, etc.

Allopurinol therapy obviously reduces the chances of uric acid nephropathy and/or arthropathy. On the other hand, it may enhance the incidence of xanthine nephrolithiasis. (1) In any case, because of the absence of HG PRTase, allopurinol cannot affect the excessive rate of purine de novo synthesis and/or establish the feedback inhibition (3).

A progressive deficiency state may play a major role in the production of symptoms. Of 23 cases so far reported, 16 were below the 3rd percentile for weight, 4 were at the 3rd percentile and 3 were between the 3rd and 10th percentiles. Phrases such as hypocaloric dwarfism, dying of inanition and difficulty in swallowing making feeding a real

problem (9) indicate that most of the patients if not all of them were severely malnourished at the time of the detection of the disease. Little attention has been paid to the significance of this deficiency state and its devastating consequences. Indeed in nine instances attempts were made to reduce blood uric acid by administration of a low purine low protein diet. Growing brain tissue which uses protein synthesis for most of its activity may find it difficult to satisfy the growth demands of its semi starved cells. Exaggerated de novo purine synthesis demanding additional molecules of glutamine, glycine and aspartic acid for the formation of each additional molecule of uric acid cannot be disputed (2). Considering that for the formation of one molecule glutamine in this disease amounts to 500-1000 mg per day. Although glutamine is not an essential amino acid the resources for its formation are not unlimited.

Recent work by Paleologos *et al* (10) shows that in rats the intracellular concentration of alanine and glutamine is markedly decreased during gluconeogenesis. The combined effects of starvation, gluconeogenesis and excessive use of glutamine for exaggerated de novo purine synthesis must deplete the amino acid pool of the cell. This intracellular deficiency state either alone or in combination with the effects of accumulated xanthines is in our opinion responsible for production of the neurological manifestations of the disease.

In support of a deficiency state and the necessity for administration of glutamine in the persistently low concentration of that substance in blood prior to administration of MSG (Table 2). Moreover the marked improvement of this therapy indicates that the deficiency state plays a major role in the production of the neurological manifestation of the disease. Further work including estimation of the intracellular concentrations of amino acids in untreated Lesch Nyhan Disease and in controls should substantiate our contention.

## SUMMARY

A typical case of Lesch Nyhan Disease which responded favorably to a high protein diet and administration of monosodium glutamate has been presented. The de novo purine synthesis has been briefly described. The semi starved condition of these patients drinking mostly juice and water has been emphasized. The role of the deficiency state in the production of symptoms and its correction have been discussed.

## ACKNOWLEDGMENT

We are indebted to Dr W. Nyhan for enzyme assay of the blood of the patient. Acknowledgment is also due to Dr J. Seegmiller for permission to reproduce a modified scheme of de novo purine biosynthesis from his article (13). We owe our thanks to Dr J. Carr for uric acid determination in routine laboratory work.

## REFERENCES

1. Green M. L., Popamoto W. Y. & Seegmiller J. P. Urinary xanthine stones—a rare complication of allopurinol therapy. *New Eng J Med* 280: 476 1969.
2. Gorman A. B. & Yu T. Uric acid metabolism in normal man and in primary gout. *New Eng J Med* 273: 232 1965.
3. Kelley W. N., Rowenbloom F. M., Miller J. & Seegmiller J. E. An enzymic basis for variation in response to allopurinol. *New Eng J Med* 278: 287 1968.
4. Kornberg A., Loberman I. & Somms E. S. Enzymic synthesis of purine nucleotides. *J Biol Chem* 215: 417 1955.
5. Lesch M. & Nyhan W. L. A familial disorder of uric acid metabolism and central nervous system function. *Amer J Med* 36: 561 1964.
6. Magasanik B. Regulation of the biosynthesis and interconversion of purine nucleotides. *Arthritis Rheum* 8: 610 1965.
7. Newton E. B. Chromatogenic tongue and its use in the determination of the uric acid of blood. *J Biol Chem* 120: 315 1937.
8. Nagel C. A. & Tyler F. H. The renal excretion of uric acid in patients with gout and in non gouty subjects. *J Clin Invest* 34: 1890 1959.
9. Nyhan W. L. Summary of clinical features. *Fed Proc* 7: 1034 1968.
10. Paleologos G., Muntwyler E. & Kewener L. Alanine and glutamine levels in rat liver tissue. A direct relationship to gluconeogenic state. *Proc Soc Exp Biol Med* 132: 210 1969.

- 11 Piez K A & Morris L A A modified procedure for automatic analysis of amino acids *Anal Biochem* 1 187 1960
- 12 Rosenbloom F M Kelley W N Miller J Henderson J F & Seegmiller J E Inherited disorder of purine metabolism *JAMA* 202 175 1967
- 13 Seegmiller J E Rosenbloom F M & Kelley W N Enzymic defect associated with a sex linked human neurological disorder and excessive purine synthesis *Science* 155 1682 1967
- 14 Speckman D H Stein W H & Moore S Automatic recording apparatus for use in the

chromatography of amino acids *Anal Chem* 30 1190 1958

Submitted Sept 17 1969

Accepted Oct 24 1969

(H G) Dept of Pediatrics  
Methodist Hospital of Brooklyn  
New York  
USA

Key words Lesch Nyhan disease, hyperuricemia, treatment with monosodium glutamate

## THE RENAL TRANSPORT OF AMINO ACIDS IN UNTREATED INFANTS WITH PHENYLKETONURIA

J BRODEHL, A GELLISSSEN and W P KAAFS

From the Department of Paediatrics (Head H Hungerford) University of Bonn Bonn Germany

Excess of a single amino acid may produce changes in the transport and metabolism of several other amino acids. This phenomenon of amino acid imbalance (9) has been considered to be effective in phenylketonuria (PKU) too and has been postulated to play an important role in the pathogenesis of this disease (10, 12, 17, 20).

The data available, however, do not suffice to prove this hypothesis. Reports of the literature are mostly concerned with the concentrations of free amino acids in the blood of phenylketonurics (10, 12, 20). Only scarce data are available for the intestinal transport of amino acids (21) and for the incorporation of amino acids into cells in the presence of high phenylalanine concentrations (3, 8, 14, 23). The renal transport of amino acids seems to be not influenced as measurements of urinary excretion in phenylketonurics demonstrated (1, 2, 12, 19). There are, however, only a few quantitative data of tubular reabsorption of amino acids in the presence of high tubular loading with phenylalanine (12) and none in infancy. Therefore, clearance studies were performed in infants with untreated phenylketonuria with simultaneous measurements of glomerular fil-

tration rate. As will be shown, the high levels of phenylalanine in the blood and tubular fluid have no significant influence on the tubular reabsorption of phenylalanine and 15 other free amino acids.

### METHODS

The studies were performed in 5 full-term infants between the ages of 4 weeks and 15 months (infants no. 2-6) and in one premature infant at the age of 3 weeks (infant no. 1). Their age, sex, birth weight, weight and body surface area at time of examination and the values of urea and phosphate clearance and urinary volume are listed in Table 1. When the diagnosis of PKU was established, the phenylalanine levels in the serum ran between 281 and 519 mg/100 ml. All infants were considered to suffer from classical PKU and were treated successfully with a low phenylalanine diet subsequently.

The clearance studies were performed just after diagnosis and before starting the treatment. The procedures of the clearance examination were the same as described elsewhere (6). The glomerular filtration rate was measured with urea. The amino acids were determined in serum and urine by ion exchange chromatography as previously described (6). 12 infants aged 16 days to 4 months served as a control group. Their data also were reported on previously (6).

### RESULTS

The results of the clearance studies in 6 infants with PKU are given in Tables 2 and 3. The data include the concentrations of free amino acids in the serum ( $P_{AA}$  expressed in mg/100 ml), the urinary excretion rate ( $U_{AA}$  in  $\mu$ g/

Supported by grants of the Deutsche Forschungsgemeinschaft. Partly presented at the Hoeselberg Symposium on Phenylketonuria 1969 and on the 6th Meeting of Deutsche Gesellschaft für Kinderheilkunde Saarbrücken 1969.



- 11 Piez, K. A. & Morris, L. A. A modified procedure for automatic analysis of amino acids *Anal Biochem* 1 187 1960
  - 12 Rosenbloom, F. M., Kelley, W. N., Miller, J., Henderson, J. F. & Seegmiller, J. E. Inherited disorder of purine metabolism *JAMA* 202 175 1967
  - 13 Seegmiller, J. E., Rosenbloom, F. M. & Kelley, W. N. Enzymic defect associated with a sex linked human neurological disorder and excessive purine synthesis *Science* 155 1682 1967
  - 14 Spackman, D. H., Stein, W. H. & Moore, S. Automatic recording apparatus for use in the chromatography of amino acids *Anal Chem* 30 1190 1958
- Submitted Sept 17 1969  
Accepted Oct 24 1969
- (H. G.) Dept of Pediatrics  
Methodist Hospital of Brooklyn  
New York  
U.S.A.
- Key words** Leish Nyhan disease; hyperuricaemia; treatment with monosodium glutamate

## THE RENAL TRANSPORT OF AMINO ACIDS IN UNTREATED INFANTS WITH PHENYLKETONURIA

J. BRODFPHIL A. GELLESSEN and W. P. A. AAS

From the Department of Paediatrics (Head H. Hungerford) University of Bonn, Bonn, Germany

Excess of a single amino acid may produce changes in the transport and metabolism of several other amino acids. This phenomenon of amino acid imbalance (9) has been considered to be effective in phenylketonuria (PKU) too and has been postulated to play an important role in the pathogenesis of this disease (10, 12, 17, 20).

The data available, however, do not suffice to prove this hypothesis. Reports of the literature are mostly concerned with the concentrations of free amino acids in the blood of phenylketonurics (10, 12, 20). Only scarce data are available for the intestinal transport of amino acid (21) and for the incorporation of amino acids into cells in the presence of high phenylalanine concentrations (3, 8, 14, 23). The renal transport of amino acids seems to be not influenced as measurements of urinary excretion in phenylketonurics demonstrated (1, 2, 12, 19). There are, however, only a few quantitative data of tubular reabsorption of amino acids in the presence of high tubular loading with phenylalanine (12) and none in infancy. Therefore clearance studies were performed in infants with untreated phenylketonuria with simultaneous measurements of glomerular fil-

tration rate. As will be shown, the high levels of phenylalanine in the blood and tubular fluid have no significant influence on the tubular reabsorption of phenylalanine and 15 other free amino acids.

### METHODS

The studies were performed in 5 full-term infants between the ages of 4 weeks and 15 months (infants no. 2-6) and in one premature infant at the age of 3 weeks (infant no. 1). Their age, sex, birth weight, weight and body surface area at time of examination and the values of urethra and phosphorus clearance and urinary volume are listed in Table 1. When the diagnosis of PKU was established, the phenylalanine levels in the serum ran between 28.1 and 51.9 mg/100 ml. All infants were considered to suffer from classical PKU and were treated successfully with a low phenylalanine diet subsequently.

The clearance studies were performed just after diagnosis and before starting the treatment. The procedures of the clearance examinations were the same as described elsewhere (6). The glomerular filtration rate was measured with inulin. The amino acids were determined in serum and urine by ion exchange chromatography as previously described (6). 12 infants aged 16 days to 4 months served as a control group. Their data also were reported on previously (6).

### RESULTS

The results of the clearance studies in 6 infants with PKU are given in Tables 2 and 3. The data include the concentrations of free amino acids in the serum ( $P_{AA}$  expressed in mg/100 ml), the urinary excretion rate ( $U_{AA}$  in  $\mu$ g/

Supported by grants of the Deutsche Forschungsgemeinschaft. Partly presented on the Heidelberg Symposium on Phenylketonuria 1969 and on the 6th Meeting of Deutsche Gesellschaft für Kinderheilkunde, Saarbrücken 1969.

Table 1 Some biographical data of the 6 infants with phenylketonuria in whom the amino acid clearance studies were performed

Infant no	Initials	Sex	Birth weight		Weight (kg)	Surface area (m <sup>2</sup> )	GFR <sup>a</sup>	Phosphate clearance <sup>b</sup>	Urinary volume <sup>c</sup>	
			(kg)	Age <sup>d</sup>			C <sub>in</sub>	(ml/min/1.73 m <sup>2</sup> )		
1	Premature	L F	♂	2.2	3 weeks	2.4	0.17	36	20.4	10.5
2		I J	♀	3.5	4 weeks	4.0	0.24	46	9.2	8.3
3		G B	♂	2.7	4 months	8.0	0.35	99	12.5	7.8
4		M P	♂	2.7	7 months	8.2	0.38	102	17.5	13.5
5		U K	♀	4.0	10 months	12.2	0.50	91	22.0	11.1
6		G W	♂	3.4	15 months	11.3	0.48	124	6.6	10.0

<sup>a</sup> At day of clearance study body surface area calculated from the nomogram according to the formula of DeLoo and DuBou.

<sup>b</sup> The values of the creatinine-clearance (C<sub>cr</sub>), phosphate-clearance and urinary volume were obtained while determining the amino acid clearance.

min/1.73 m<sup>2</sup>) the endogenous renal clearance rates (C<sub>cr</sub> in ml/min/1.73 m<sup>2</sup>) and the percentage tubular reabsorption of amino acids (%T<sub>cr</sub>). The mean values of the 5 fullterm infants (infant no 2-6)—the premature infant no 1 was excluded since prematures are known to have renal hyperaminoaciduria—are compared with the mean values of 12 normal infants (aged 16 days to 4 months) which is depicted in Figs 1-4.

The serum concentration of phenylalanine ran between 28.1 and 51.9 mg/100 ml, with a mean value of 35.9 mg/100 ml (Table 2). The concentrations of most other amino acids tended to be lower in phenylketonurics than in the normals (Fig 1). This was especially valid for proline, alanine, methionine, ornithine and histidine which had significantly de-

creased mean values ( $p < 0.02$ ). The other amino acids did not show significant changes.

The urinary excretion showed the typical high values for phenylalanine (Table 2 and Fig 2). All other amino acids however were excreted in approximately the same or slightly lower amounts than in the normals.

The values of the endogenous clearance rates were normal for phenylalanine and all other amino acids measured (Table 3 and Fig 3). The mean values of the 5 infants with PKU even tended to be lower than those of the normals. This however can easily be explained by the fact that the mean age of the phenylketonurics was somewhat higher than that of the control group.

The percentage tubular reabsorption of phenylalanine and the other free amino acids are listed in Table 3 and depicted in Fig 4.

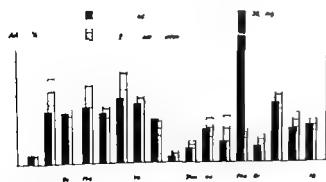


Fig 1 The concentrations of free amino acids in the serum of five infants with untreated PKU (mean values - black columns) in comparison with the values of normal infants (mean values and standard deviation - white columns). The decrease of proline, alanine, methionine, ornithine and histidine are statistically significant ( $p < 0.02$ ).

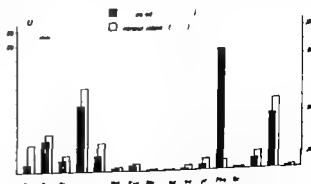


Fig 2 The urinary excretion of free amino acids in infants with untreated PKU (mean values - black columns) and in normal infants (mean values - white columns).

The mean values of percentage tubular reabsorption were within the normal range for all most all amino acids measured. The only exception seems to be phenylalanine which was reabsorbed even more completely in the phenylketonurics than in the normals.

## DISCUSSION

To our knowledge direct measurements of renal clearance and tubular reabsorption of amino acids in PKU were reported only by Efron and coworkers (12). Their patients were 7 and 9 years old, one was untreated and the other one under low phenylalanine diet for 2 weeks. Other authors calculated the clearance rates of phenylalanine and other amino acids without simultaneous measurements of glomerular filtration rates (1, 19). Our results confirm the findings of these authors, i.e. the endogenous clearance rates of phenylalanine and other amino acids remain approximately the same despite the enormous elevation of blood phenylalanine levels in PKU.

Our results also confirm the observations that several amino acids in the blood are lower in PKU than in normals (10, 12, 17, 20). These findings have raised speculations concerning a possible pathogenetic role of amino acid imbalance in this disease. The data however are not uniform. In Table 4 those free amino acids are listed which were compared in the blood of phenylketonurics and normals by 4 groups of investigators. As can be seen a uniform statistically significant decrease ( $p < 0.05$ ) was found only for alanine. The data of the other amino acids measured are variable.

It is our opinion that the decrease of amino acid in PKU is just a symptom of the disease and not a cardinal pathogenetic mechanism. It merely reflects different rates of amino acid uptake and metabolism by the tissues and possibly different rates of intestinal resorption (21) which may be the consequence of various enzyme stimulations by the high phenylalanine levels (22). It seems to be unspecific since

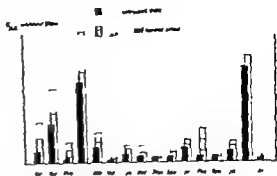


Fig 3 The endogenous renal clearance rate of free amino acids in untreated PKU (mean values - black columns) and normal infants (mean values and standard deviation - white columns).

lowering of several amino acids in the blood can be provoked by many factors including painful stimuli (30), saline or glucose infusion (5), imbalanced or deficient nutrition (13, 37), hormonal actions (18, 22, 25, 39), vitamin deficiency (16) and severe renal loss of amino acids (4). The reported effect of phenylalanine infusion also may be unspecific (12). An oral loading with leucine (24, 36), phenylalanine (10) and histidine (15) also produces comparable declines of several other amino acids in the blood.

For the renal physiologist there are some more aspects to be discussed. The tubular transport system for phenylalanine reabsorption seems to be very highly developed since a 30 to 50 fold overloading is handled as suffi-

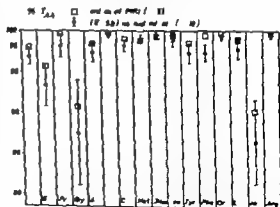


Fig 4 The percentage tubular reabsorption of amino acids in infants with untreated PKU (mean values - white squares) and in normal infants (mean values and standard deviation - black dots with vertical lines).

Table 1 Some biographical data of the 6 infants with phenylketonuria in whom the amino acid clearance studies were performed

Infant no	Initials	Sex	Birth weight (kg)	Age	Weight <sup>a</sup> (kg)	Surface area (m <sup>2</sup> )	GFR <sup>b</sup> C <sub>in</sub> (ml/min/1.73 m <sup>2</sup> )	Phosphate clearance <sup>b</sup>	Urinary volume <sup>b</sup>	
1	Premature	L F	♀	2.2	3 weeks	2.4	0.17	56	20.4	10.5
2		J J	♀	3.5	4 weeks	4.0	0.24	46	9.2	8.3
3		G B	♂	2.7	4 months	8.0	0.35	99	12.5	7.8
4		M P	♂	2.7	7 months	8.2	0.38	102	17.5	13.5
5		U K	♀	4.0	10 months	12.2	0.50	91	22.0	6.8
6		G W	♀	3.4	15 months	11.3	0.49	124	6.6	10.0

<sup>a</sup> At day of clearance study body surface area calculated from the nomogram according to the formula of Dubois and DuBois

<sup>b</sup> The values of the inulin-clearance (C<sub>in</sub>), phosphate-clearance and urinary volume were obtained while determining the amino acid clearance

min/1.73 m<sup>2</sup>) the endogenous renal clearance rates (C<sub>in</sub> in ml/min/1.73 m<sup>2</sup>) and the percentage tubular reabsorption of amino acids (% T<sub>in</sub>). The mean values of the 5 fullterm infants (infant no 2-6)—the premature infant no 1 was excluded since prematures are known to have renal hyperaminoaciduria—are compared with the mean values of 12 normal infants (aged 16 days to 4 months) which is depicted in Figs 1-4.

The serum concentration of phenylalanine ran between 281 and 519 mg/100 ml with a mean value of 359 mg/100 ml (Table 2). The concentrations of most other amino acids tended to be lower in phenylketonurics than in the normals (Fig. 1). This was especially valid for proline, alanine, methionine, ornithine and histidine which had significantly decreased mean values ( $p < 0.02$ ). The other amino acids did not show significant changes.

The urinary excretion showed the typical high values for phenylalanine (Table 2 and Fig. 2). All other amino acids, however, were excreted in approximately the same or slightly lower amounts than in the normals.

The values of the endogenous clearance rates were normal for phenylalanine and all other amino acids measured (Table 3 and Fig. 3). The mean values of the 5 infants with PKU even tended to be lower than those of the normals. This, however, can easily be explained by the fact that the mean age of the phenylketonurics was somewhat higher than that of the control group.

The percentage tubular reabsorption of phenylalanine and the other free amino acids are listed in Table 3 and depicted in Fig. 4.

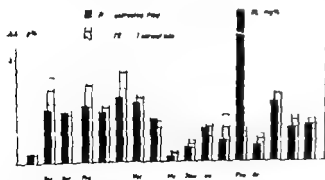


Fig. 1 The concentrations of free amino acids in the serum of five infants with untreated PKU (mean values—black columns) in comparison with the values of normal infants (mean values and standard deviation—white columns). The decrease of proline, alanine, methionine, ornithine and histidine are statistically significant ( $p < 0.02$ ).

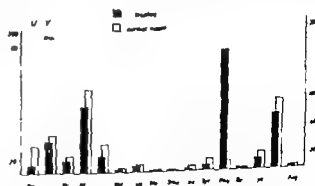


Fig. 2 The urinary excretion of free amino acids in 10 infants with untreated PKU (mean values—black columns) and in normal infants (mean values—white columns).

Table 3 Values of endogenous clearance rates of free amino acids ( $C_{AA}$  in ml/min/1.73 m<sup>2</sup>) and of percentage tubular reabsorption (%  $T_{AA}$ ) in 6 boys with untreated phenylketonuria

The mean values of values no. 2-6 are depicted in Page 3 and 4. The control values are derived from 12 infants aged 16 days to 4 months (6)

Amino acids	Endogenous clearance rates ml/min/1.73 m <sup>2</sup>										Percentage tubular reabsorption									
	Infant no.										Controls									
	1	2	3	4	5	6	Controls				1	2	3	4	5	6	Controls			
							$\bar{V}$	$\pm$	SD								$\bar{V}$	$\pm$	SD	
Thr	2.99	4.64	—	—	—	0.44	2.00	1.39			—	89.91	—	97.61	—	96.63	96.97	1.06		
Ser	—	—	0.4	—	2.91	1.33	4.06	1.94			—	89.91	—	99.76	—	98.93	93.41	2.65		
Pro	—	—	0.4	—	0.46	—	7.79	0.66			—	23.16	—	97.37	—	99.30	98.25	1.39		
Gly	4.80	11.43	1.02	—	6.74	4.67	3.24	3.24			—	96.46	—	98.09	—	96.23	87.57	4.44		
Ala	0.44	0.23	0.34	—	1.47	0.36	1.63	0.75			—	94.41	—	98.76	—	99.45	97.34	1.10		
Val	2.23	0.87	0.34	—	0.24	0.13	0.29	0.1			—	94.41	—	99.66	—	99.90	99.50	0.42		
Met	0.30	0.38	—	—	0.34	—	1.07	0.27			—	94.41	—	—	—	—	98.22	0.72		
Ileu	—	0.18	—	—	—	—	0.79	0.56			—	99.91	—	—	—	—	98.79	0.90		
Leu	—	0.33	—	—	0.28	0.39	0.70	0.16			—	98.83	—	—	—	—	99.43	0.13		
Tyr	2.40	1.21	1.08	—	1.33	0.87	1.79	0.41			—	97.37	—	98.91	—	99.50	97.23	1.14		
Phe	0.76	0.37	0.40	—	0.48	0.32	1.74	0.91			—	99.0	—	99.60	—	99.74	97.36	1.01		
Orn	0.67	0.37	0.35	—	0.52	0.45	0.29	0.14			—	99.40	—	99.65	—	99.64	99.37	0.27		
Lys	2.92	1.21	0.61	—	1.02	0.57	1.29	0.37			—	97.37	—	99.38	—	99.57	97.80	1.14		
His	2.92	9.27	5.01	—	8.80	6.47	8.52	4.46			—	79.85	—	94.91	—	94.78	96.62	5.67		
Arg	—	0.13	0.19	—	0.24	0.16	0.36	0.08			—	99.78	—	99.81	—	99.74	99.41	0.21		

Table 2. Values of serum concentrations of free amino acids ( $P_{AA}$  in mg/100 ml) and of urinary excretion rates ( $U_{AA}$  in  $\mu$ g/min/1.73  $m^2$ ) in 6 infants with untreated phenylketonuria

The mean values of infant no. 2-6 are depicted in Figs 1 and 2. The control values are derived from 12 infants aged 18 days to 4 months (6)

Amino acids	Serum concentrations mg/100 ml						Urinary excretion rate $\mu$ g/min/1.73 $m^2$											
	Infant no						Controls						Infant no					
	1	2	3	4	5	6	$\bar{x}$	$\pm$ SD	1	2	3	4	5	6	$\bar{x}$	$\pm$ SD	1	2
Thr	4.00	1.30	—	1.51	1.27	1.72	1.51	0.39	119.6	60.3	14.5	58.3	—	—	67	39.3	—	—
Ser	—	—	2.35	1.52	1.04	—	1.50	0.29	—	38.8	35.8	0.0	—	20.0	—	19.7	—	—
Gly	1.72	1.66	1.93	1.41	1.15	—	1.18	0.60	348.1	38.8	5.6	0.0	4.8	—	—	54.4	—	—
Ala	2.12	1.71	2.34	2.03	1.60	1.77	1.60	0.26	101.7	189.8	50.2	101.6	77.5	55.1	23.6	23.3	—	—
Val	1.66	1.72	1.86	2.23	1.52	1.30	1.60	0.47	107.7	27.9	23.3	25.9	25.1	10.0	119.9	59.9	—	—
Cys	1.20	1.38	—	1.18	1.24	—	1.30	0.45	26.8	4.7	6.3	2.2	3.6	1.7	40.9	14.6	—	—
Met	0.30	0.20	0.18	0.18	0.12	—	0.18	0.21	0.9	12.1	7.7	6.4	4.8	—	5.6	2.8	—	—
Ileu	0.46	0.32	0.54	0.57	0.43	0.31	0.43	0.04	—	1.0	nd	nd	—	—	10.6	3.7	—	—
Leu	0.82	0.70	1.97	0.91	0.72	0.57	0.72	0.11	—	0.6	0.9	4.3	1.2	1.7	1.4	1.1	—	—
Tyr	1.34	0.80	0.78	0.69	0.45	0.38	0.57	0.28	32.1	3.7	—	2.2	2.4	3.3	1.3	1.0	—	—
Phe	31.28	51.86	33.95	35.92	33.96	28.05	33.96	0.38	—	9.7	8.4	8.6	6.0	3.3	16.3	8.4	—	—
Orn	0.72	0.59	0.62	0.28	0.46	0.38	0.46	0.16	237.4	189.8	134.4	280.9	162.2	90.1	14.4	7.9	—	—
Lys	1.65	2.05	1.99	1.70	1.40	1.57	1.66	0.38	4.8	2.2	2.2	2.2	2.4	1.7	2.5	1.0	—	—
His	0.88	1.08	1.00	1.15	1.07	0.62	1.07	0.41	49.1	25.0	12.2	13.0	14.3	8.3	25.1	10.3	—	—
Arg	—	1.00	1.52	0.85	0.99	1.06	1.22	0.22	78.5	100.0	50.2	101.6	94.2	40.1	99.5	48.8	—	—
	—	—	—	—	—	—	1.09	0.17	6.8	3.2	2.9	2.2	2.2	1.7	3.9	1.2	—	—

nd = not detectable





Table 2. Values of serum concentrations of free amino acids ( $P_{AA}$  in mg/100 ml) and of urinary excretion rates ( $U_{AA}$  in  $\mu$ g/min/1.73  $m^2$ ) in 6 infants with untreated phenylketonuria

The mean values of infant no. 2-6 are depicted in Figs 1 and 2. The control values are derived from 12 infants aged 16 days to 4 months (6)

Amino acids	Serum concentrations mg/100 ml						Urinary excretion rate $\mu$ g/min/1.73 m <sup>2</sup>											
	Infant no						Controls						Infant no					
	1	2	3	4	5	6	$\bar{y}$	$\pm$ s.d.	1	2	3	4	5	6	$\bar{y}$	$\pm$ s.d.		
Tyr	4.00	1.30	—	1.51	—	1.72	1.51	0.39	—	119.6	—	14.5	—	—	67	39.3	19.7	
Ser	—	—	2.35	1.52	1.04	—	1.50	0.29	—	60.3	35.8	58.3	50.1	—	20.0	54.4	21.8	
Gly	2.12	1.66	1.93	1.41	1.15	—	1.38	0.60	—	148.1	56	0.0	4.8	—	—	23.6	23.3	
Ala	2.12	1.71	2.34	2.03	1.60	1.77	1.60	0.47	101.7	189.8	50.2	101.6	77.5	55.1	119.9	59.9	59.9	
Val	1.66	1.72	1.86	2.23	1.52	1.30	1.69	0.45	101.7	27.9	23.3	25.9	25.1	10.0	40.9	14.6	14.6	
Cys	1.20	1.38	—	1.18	1.4	—	—	—	10.7	4.7	6.3	2.2	3.6	1.7	5.6	2.8	2.8	
Ileu	0.30	0.20	0.18	0.18	0.12	—	0.18	0.21	26.8	12.1	7.7	6.4	4.8	—	—	10.6	3.7	
Leu	0.46	0.32	0.54	0.57	0.43	—	0.31	0.04	0.9	0.6	0.9	4.3	1.2	1.7	1.1	1.1	1.1	
Tyr	0.82	0.70	1.97	0.91	0.72	0.57	0.72	0.28	—	—	—	2.2	2.4	3.7	1.3	1.0	1.0	
Phe	1.34	0.80	0.78	0.69	0.45	0.38	0.98	0.38	32.1	9.7	8.4	8.6	6.0	3.3	16.3	8.4	8.4	
Orn	31.28	51.86	33.95	35.93	33.96	28.05	0.87	0.16	237.4	189.8	134.4	280.9	162.2	90.1	14.4	7.9	7.9	
Lys	0.72	0.59	0.62	0.28	0.46	0.38	0.66	0.14	4.8	2.2	2.2	2.2	2.4	1.7	2.5	1.0	1.0	
His	1.65	2.05	1.99	1.70	1.40	1.57	1.97	0.41	49.1	25.0	12.2	13.0	14.3	8.3	25.1	10.3	10.3	
Arg	0.88	1.08	1.00	1.15	1.07	0.62	1.22	0.22	78.5	100.0	50.2	101.6	94.2	40.1	99.5	48.8	48.8	
—	—	1.00	1.42	0.85	0.99	1.06	1.09	0.17	6.8	3.2	2.9	2.2	2.4	1.7	3.9	1.2	1.2	

n.d. = not detectable

n.d. = not detectable

alanine—as could be suggested from the findings of van Sikkelenburg (38)—or that some phenylalanine is disintegrated or bound during the passage through the tubular canals. Our present methods do not allow us to investigate this further.

### SUMMARY

In 5 full-term infants aged 4 weeks to 15 months and in one premature infant aged 3 weeks with untreated phenylketonuria renal clearance studies were performed in order to determine the renal transport of phenylalanine and 15 other free amino acids. The data show that the high elevation of phenylalanine in the blood has no significant influence on the clearance rates and percentage tubular reabsorption of all amino acids measured. Phenylalanine seems to be reabsorbed even more completely in PKU than in normals. It is concluded that the tubular transport system for phenylalanine is characterized by a high specificity and high capacity which is in the contrast to the tubular transport system for the amino acids and glycine.

In the serum the concentrations of some amino acids are lower in the presence of high phenylalanine levels. This finding already reported by other authors before is considered to be unspecific and without any significance for the pathogenesis of this disease.

### ACKNOWLEDGEMENTS

We gratefully acknowledge the technical and secretarial assistance of Miss A. Jaki, Mrs G. Service, Miss C. Horn and Miss M. Kowalski (R.N.).

### REFERENCES

1. Averbach V. H., DeGeorge A. M. & Carpenter G. G. Phenylalaninemia. In W. L. Nyhan (ed.) *Amino acid metabolism and genetic disease*. McGraw-Hill Book Comp. New York Toronto London and Sydney 1967 p. 11.
2. Berry H. A., Umberger H. & Livingston B. Excretion of phenylalanine by normal children and by patients with phenylketonuria. *J. Pediatr.* 63: 954 1963.
3. Bickel I. J., Kennedy J. P. & Quastel J. H. Phenylketonuria: inhibition of tyrosine metabolism in the liver. *Nature* 179: 114 1957.

4. Braun L., Brodehl J. & Gellman A. Renal glucose losing syndrome. *XII International Congress of Pediatrics Mexico 1968 Abstracts Vol III* p. 310.
5. Brodehl J. Der renale Transport der Aminosäuren im Säuglings- und Kindesalter. *Beihft. Arch. Kinderheilkunde* No. 61. Ferdinand Enke Verlag Stuttgart 1969.
6. Brodehl J. & Gellman A. Endogenous renal transport of free amino acids in infancy and childhood. *Pediatrics* 42: 395 1968.
7. Brodehl J., Gellman A. & Kowalski S. Isolierter Defekt der tubulären Aminosäureabsorption in einer Familie mit idiopathischem Hypoparathyroidismus. *Klin. Wochschr.* 45: 38 1967.
8. Chirigos M. A., Greenap P. & Udenfied S. The uptake of tyrosine by rat brain *in vivo*. *J. Biol. Chem.* 235: 2075 1960.
9. Christensen H. N. Metabolism of amino acids and proteins. *Ann. Rev. Biochem.* 22: 233 1953.
10. Colombo J. B. & Vassella P. Das Aminosäurespektrum im Plasma bei der Phenylketonurie. Beeinflussung durch L-Phenylalanin und L-Tyrosinbelastung. *Klin. Wochschr.* 46: 1215 1968.
11. DeVries A., Kowalski S., Lazebnik J., Frank M. & Ojeda M. Glycemia: a hereditary disorder associated with nephrolithiasis. *Amer. J. Med.* 23: 408 1957.
12. Edeon M. L., Song Kwang E., Vancorp J. & Fellers F. X. Effect of elevated plasma phenylalanine levels on other amino acids in phenylketonuric and normal subjects. *J. Pediatr.* 74: 399 1969.
13. Fehg P., Owen O. H., Wahren J. & Cahall G. P. In Amino acid metabolism during prolonged starvation. *J. Clin. Invest.* 48: 584 1969.
14. Garroff D., Jung W. & Udenfied S. The uptake of tyrosine by rat brain *in vivo*. *J. Biol. Chem.* 236: 1773 1961.
15. Holman J. B. The effect of a histidine load on plasma levels and renal clearances of other amino acids. *Clin. Chem. Acta* 21: 241 1968.
16. Karm W. P. & Brodehl J. Verstoffwechselung und renaler Aminosäuretransport beim Vitamin C Mangel (Vorderhornsche Erkrankung). 67. Tagung Deutsche Gesellschaft Kinderheilkunde Saarbrücken 1969. *Monat. Kinderheilk.* in press.
17. Karm W. P. Phenylketonuria. In J. B. Stanbury, J. B. Wyngaarden & D. S. Fredrickson (eds.) *The metabolic basis of inherited disease*. McGraw-Hill Comp. New York Toronto London and Sydney 1966. 2nd ed. p. 238.
18. Landau R. L. & Ingber A. Effect of glucose on catabolic rates of several free amino acids in plasma. *Metabolism* 18: 263 1969.
19. Linoweb F. & Ehrlich M. Die renalen und pararenalen Störungen des Aminosäurestoffwechsels bei Phenylalanin-ämie Ernährung. *Klin. Wochschr.* 38: 904 1960.
20. — Zur Pathogenese des Schwachsinn bei Phenylketonurie. *Klin. Wochschr.* 40: 225 1962.

Table 4 Comparison of amino acid concentrations of blood in phenylketonurics and normal individuals performed by 4 groups of investigation

All statistically significant differences (with  $p$  values less than 0.05 [ $p < 0.05$ ]) are listed  
 N = no difference ↓ = decrease ↑ = increase — = not determined

	Glu Thr Glu NH <sub>2</sub> Pro Gly Ala Val Cys Met Ileu Leu Tyr Orn Lys His Arg													
Linneweh & Ehrlich 1962														
PKU $n=14$		—	—	—	—	—	—	—	—	—	—	—	—	—
normals $n=10$	↓													
Colombo & Visselli 1968														
PKU $n=6$	—	↓	—	N	N*	↓	N	N	N	↓	N*	N*	—	N*
normals $n=8$														
Efron et al 1969														
PKU $n=20$	↓	N	↓	↓	N	↓	↓	↓	↓	↓	↓	↓	—	—
normals $n=10$														
Brodehl et al 1970														
PKU $n=5$	—	—	—	↓	N	↓	N	N	↓	N	N	N	↓	N
normals $n=12$														

\* Differences with  $p$  values in the range of 0.05 to  $<0.1$  which were considered as significant by Colombo & Visselli are rated here as N = no differences for reasons of comparison

ciently as the physiological load. This is true not only for older children and adults but for infants also as our results demonstrate. It seems quite impossible to reach a tubular maximum for phenylalanine reabsorption in man. It was neither reached in dogs in an early study by Russo and coworkers (29).

The high tubular load with phenylalanine does neither produce any competitive nor inhibitory effect on the transport of other amino acids. In analogy to the effects of lysine infusion (26) one would have expected that high phenylalanine loads would possibly compete with the transport of other neutral amino acids shared by the same transport system. This however cannot be demonstrated.

According to Scriver (34, 35) and Rosenberg (28) and their coworkers there is much evidence that there exist at least two transport systems for reabsorption of a single amino acid in the renal tubules. The one system is reported to have a high substrate specificity and a low capacity and the other one a low or group specificity but a high capacity for transport. Both systems are controlled separately by different genes as studies of inborn errors—classical cystinuria (27) and isolated hypercystinuria (7), iminoglycinuria (31) and glycinuria (11)—revealed.

Our findings suggest as Scriver (32) has already pointed out that the tubular reabsorption of phenylalanine is ruled by a transport system which is highly specific and also has high capacity. This is in contrast to the system described for the amino acids and glycine (3). The transport system for phenylalanine demonstrates an early ontogenic maturation: the results in the premature and young infants prove it. It is concluded that there are differences between the various tubular transport systems and that these have to be investigated for each individual amino acid separately.

Finally it is astonishing that the percentage tubular reabsorption of phenylalanine ( $\% T_{ra}$ ) seems to be more complete in PKU than normals. In the normal infant  $\% T_{ra}$  is in the range of  $97.36 \pm 1.01$  (6). In the full-term infants with PKU the values ranged between 99.20 and 99.74° and in the 3 weeks premature infant with PKU the  $\% T_{ra}$  was 98.64°. It is surprising that this more complete reabsorption is a constant finding in 6 infants examined. Since we have no knowledge of a systematic error in the analytical procedures it may be speculated that there either exist some protein binding of phenylalanine in high blood concentrations of phenyl-

alanine—as could be suggested from the findings of van Stekelenburg (38)—or that some phenylalanine is deaminated or bound during its passage through the tubular canals. Our present methods do not allow to investigate this further.

### SUMMARY

In 5 full-term infants aged 4 weeks to 15 months and in one premature infant, aged 3 weeks with untreated phenylketonuria renal clearance studies were performed in order to determine the renal transport of phenylalanine and 15 other free amino acids. The data show that the high elevation of phenylalanine in the blood has no significant influence on the clearance rates and percentage tubular reabsorption of all amino acids measured. Phenylalanine seems to be reabsorbed even more completely in PKU than in normals. It is concluded that the tubular transport system for phenylalanine is characterized by a high specificity and high capacity which is in the contrast to the tubular transport system for the amino acids and glycine.

In the serum the concentrations of some amino acids are lower in the presence of high phenylalanine levels. This finding already reported by other authors before is considered to be unspecific and without any significance for the pathogenesis of this disease.

### ACKNOWLEDGEMENTS

We gratefully acknowledge the technical and nursing assistance of Miss A. Jaki, Mrs O. Herron, Miss C. Kern and Miss H. Kowalski (R.N.).

### REFERENCES

1. Auerbach V H, DeGeorge A M & Carpenter G G. Phenylketonuria. In W L Nyhan (ed) *Amino acid metabolism and genetic variations*. McGraw Hill Book Comp. New York Toronto London and Sydney 1967 p 11.
2. Berry H A, Umbarger B & Livingston B. Excretion of phenylalanine by normal children and by patients with phenylketonuria. *J Pediatr* 63 954 1963.
3. Beckis J J, Kennedy J H & Quastel J H. Phenylalanine inhibition of tyrosine metabolism in the liver. *Nature* 179 1124 1957.
4. Braun L, Brodehl J & Gellissen A. Renal glucose losing syndrome. XII International Congress of Pediatrics Mexico 1968 Abstracts Vol III p 310.
5. Brodehl J. Der renale Transport der Aminosäuren im Säuglings- und Kindesalter. *Beihft Arch Kinderheilkunde* No 61 Ferd Enke Verlag Stuttgart 1969.
6. Brodehl J & Gellissen A. Endogenous renal transport of free amino acids in infancy and childhood. *Pediatrics* 42 395 1968.
7. Brodehl J, Gellissen A & Kowalski S. Isobarter Defekt der tubulären Cystin-Rückresorption in einer Familie mit idiopathischem Hyponatrikalysemia. *Klin Wochr* 44 18 1967.
8. Churgin M A, Griesgard P & Udenfjord D. The uptake of tyrosine by rat brain *in vivo*. *J Biol Chem* 243 2075 1968.
9. Christensen H N. Metabolism of amino acids and proteins. *Ann Rev Biochem* 22 233 1953.
10. Colombo J B & Vassella F. Das Aminosäuren-spektrum im Plasma bei der Phenylketonurie beeinflusst durch L-Phenylalanin und L-Thyrosinbelastung. *Klin Wochr* 46 1215 1968.
11. DeVries A, Kowalski S, Litzental J, Frank M & Dykchalski M. Glycinuria: a hereditary disorder associated with nephrolithiasis. *Amer J Med* 23 408 1957.
12. Efron M L, Song Kung H, Vissocci J & Follery F X. Effect of elevated plasma phenylalanine levels on other amino acids in phenylketonuric and normal subjects. *J Pediatr* 74 399 1969.
13. Feig P, Owen O F, Wahren J & Cahill G F Jr. Amino acid metabolism during prolonged starvation. *J Clin Invest* 44 584 1969.
14. Garoff G, Song W & Udenfjord S. The uptake of tyrosine by rat brain *in vitro*. *J Biol Chem* 246 1771 1961.
15. Hanson J B. The effect of a histidine load on plasma levels and renal clearances of other amino acids. *Clin Chim Acta* 21 241 1968.
16. Vass W P & Brodehl J. Nierenfunktion und renaler Aminosäuretransport beim Vitamin C Mangel (Möller-Burkowsche Erkrankung). 67 Tagung Deutsche Gesellschaft Kinderheilkunde Garmisch 1969. *Mittr Kinderheilk*. In press.
17. Axel W E. Phenylketonuria. In J B Stanbury J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease*. McGraw Hill Comp. New York Toronto London and Sydney 1966 2nd ed p 239.
18. Landau E L & Lippold K. Effect of glucose on concentration of several free amino acids in plasma. *Metabolism* 18 65 1969.
19. Lissens J & Elstich V. Die renalen und peripheren Störungen des Aminosäuren-Stoffwechsels bei Phenylalanin-ämie. *Klin Wochr* 38 904 1960.
20. — Zur Pathogenese des Schwachsinns bei Phenylketonurie. *Klin Wochr* 40 225 1962.

- 21 Linneweß F, Ehrlich M, Graul E H & Hundscheidt H. Über den Aminosäuren Transport bei phenylketonurischer Oligophrenie. *Klin Wochr* 41: 253 1963
- 22 Meister A. *Biochemistry of the amino acids*. Academic Press, New York and London 1965 2nd ed.
- 23 Neame K B. Phenylalanine as inhibitor of transport of amino acids in the brain. *Nature* 192: 173 1961
- 24 Nyhan W L, Borden M & Chiklis B. Idiopathic hyperglycinemia. A new disorder of amino acid metabolism. II. The concentration of other amino acids in the plasma and their modifications by the administration of leucine. *Pediatrics* 27: 539 1961
- 25 Riggs T R & Waller L M. Growth hormone stimulation of amino acid transport into rat testes *in vivo*. *J Biol Chem* 235: 3603 1960
- 26 Robson E H & Rose O A. The effect of intravenous lysine on the renal clearances of cystine, arginine and ornithine in normal subjects in patients with cystinuria and Fanconi syndrome and their relatives. *Clin Sci* 16: 75 1957
- 27 Rosenberg L E. Cystinuria: genetic heterogeneity and allelism. *Science* 154: 1341 1966
- 28 Rosenberg L E, Albrecht I & Segal S. Lysine transport in human kidney: evidence for two systems. *Science* 155: 1426 1967
- 29 Russo H F, Wright L D, Slaggs H R, Tillson E A & Beyer K H. Renal clearances of essential amino acids: threonine and phenylalanine. *Proc Soc Exper Biol & Med* 65: 215 1947
- 30 Rouser G, Jelinek B, Samuels A J & Kinsgus A. Free amino acids in the blood of man and animals. I. Method of study and the effects of venipuncture and food intake on blood free amino acids. In J T Holden (ed.) *Amino acid pools*. Elsevier Publ Comp, Amsterdam, London and New York 1962 p 350
- 31 Scriver C R. Renal tubular transport of proline, hydroxyproline and glycine. III. Genetic basis for more than one mode of transport in human kidneys. *J Clin Invest* 47: 823 1968
- 32 —. Use of human genetic variation to study membrane transport of amino acids in kidney. *Amer J Dis Child* 117: 4 1969
- 33 Scriver C R, Efron M L & Schafer I L. Renal tubular transport of proline, hydroxyproline and glycine in health and in familial hyperprolinemia. *J Clin Invest* 43: 374 1964
- 34 Scriver C R & Muhyiddin F M. Amino acid transport in kidney. Heterogeneity of amino acid butyric uptake. *J Biol Chem* 243: 3707 1968
- 35 Scriver C R & Wilson O H. Amino acid transport: evidence for genetic control of two types in human kidney. *Science* 155: 1478 1967
- 36 Snyderman S E, Cusworth D C, Rottman E & Holt L E Jr. Amino acid interrelationships: The effect of variations in leucine intake. *Proc Soc* 18: 546 1949
- 37 Snyderman S E, Holt L E, Norton P W, Rottman E & Phisalkar S V. The plasma aminoogram. I. Influence of the level of protein intake and a comparison of whole protein and amino acid diets. *Pediat Res* 2: 131 1968
- 38 Stekelburg G J van & Desplanque J. Di-proteinization by ultrafiltration with centrifuge force. In *Techniques in amino acid analysis*. Technicon Instruments Comp., Chertsey, England 1966 p 51
- 39 Zimmermann Telschow H, Bethge H, Herberich L & Zimmermann H. Untersuchungen an Menschen über die Veränderungen der Aminosäuren im Plasma im Verlauf des Insulin-Schocks. *Texte Klin Wochr* 45: 768 1967

Submitted Oct 21 1969

Accepted Nov 25 1969

(J B) Kinderklinik der Universität  
Adenauer Allee 119  
D 5300 Bonn  
B III D

**Key words:** Phenylketonuria, amino acid excretion, amino acid clearance, tubular reabsorption of amino acids.

## PRIMARY HYPERPARATHYROIDISM IN CHILDREN

### *Brief Review of the Literature and a Case Report*

ARNE BJERNULF KERSTIN HALL, IRÈNE SJÖGREN and IVAR WERNER

*From the Departments of Medicine and Paediatrics University Hospital Uppsala  
and the Department of Endocrinology Karolinska Hospital Stockholm Sweden*

Primary hyperparathyroidism is a rare disease in children and only a few reports concerning its clinical manifestations have been published in the paediatric literature.

In 1960 Nolan *et al* (31) reviewed briefly the 23 previously reported cases. Wilkins (49) described the symptoms under four main headings: 1) those due to hypercalcaemia; 2) those resulting from the increased excretion of calcium and phosphorus in the urine; 3) those related to changes in the skeleton; and 4) metastatic calcification in soft tissues but he pointed out that under certain circumstances the typical symptoms and chemical changes of primary hyperparathyroidism may be altered.

On the basis of the clinical picture in a 13-year-old boy and an analysis of 42 paediatric patients with hyperparathyroidism reported in the literature we aim in this paper to elucidate the difficulties of differential diagnosis in this disease in children. We would point out in particular that primary hyperparathyroidism should be considered in patients with diffuse symptoms such as general fatigue and weakness, dizziness, anorexia, gastrointestinal bleeding, cardiac arrhythmia or renal colic. When

kept in mind the diagnosis is usually not difficult to establish and primary hyperparathyroidism is fortunately one of those conditions which often can be cured by adequate surgical therapy. However, this diagnosis should be considered even when there is no large increase in the serum calcium level as will be shown below.

### CASE REPORT

P. L., a 13-year-old boy, was reportedly in good health until one year prior to admission to hospital when he developed an abnormal increase in weight and daily diffuse headaches over the forehead. Between the ages of 11 and 13 years he had grown only 3 cm in height but had almost doubled his weight. The headaches, which were relieved by salicylates, were not preceded by any prodromal symptoms. There were no abdominal pains, no gastrointestinal or micturitional disturbances, no increase in thirst and no polyuria; the stools and urine seemed to be normal and there were no cardiac symptoms.

Previously the boy had had measles, rubella, pertussis and chickenpox without complications. He was the youngest of three brothers and was born as a normal delivery after a normal pregnancy, birth weight 4070 g. The development was normal and he had no difficulties at school. His mother died of systemic lupus erythematosus when he was 12 years of age. Otherwise the family was healthy and no tuberculous or allergic endocrine or nervous diseases were known among any of his relatives.

The patient was admitted immediately as an inpatient for examination at the Paediatric Department of the University Hospital in Uppsala, whence he was referred to the Department of Endocrinology of the

Department of Medicine University Hospital Uppsala

Department of Endocrinology Karolinska Hospital Stockholm

Department of Paediatrics University Hospital Uppsala

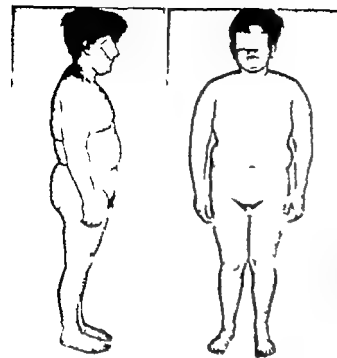


Fig. 1 A 13-year-old boy with obesity and primary hyperparathyroidism

Karolinska Hospital in Stockholm where hypophyseal and adrenal origins of the symptoms were excluded. Because of slight hypercalcaemia hyperparathyroidism was considered possible. The examination was continued at the Department of Medicine of the University Hospital in Uppsala and operation with removal of a parathyroid adenoma was performed at the Department of Surgery of the latter hospital.

#### Preoperative physical examination

When first examined the patient was 13 years old, an apparently intelligent boy with a height of 139 cm (normal 140–165 for this age) and weight 56 kg (normal 25–40 kg for this height). There was pronounced truncal obesity (Fig. 1) with an accumulation of fat between the scapulae and up to the back of the neck and stripe over the hips. No muscular atrophy was apparent but the finger joints allowed considerable hyperextension. The blood pressure was 130/100 mm Hg. The teeth, gums, mouth and throat, thyroid gland, heart, lungs, abdomen and neurological status were normal. Incipient hair growth was noted over the pubic region and in the axillae. The penis, scrotum, testes and prostate gland seemed to be rather small for his age.

#### Laboratory findings

Haemoglobin 15.5 g/100 ml. Erythrocytes 4.6 million/mm<sup>3</sup>. Leucocytes 6000/mm<sup>3</sup>. Erythrocyte sedimentation rate 5–12 mm/h. Total serum protein (v. Sjölye) 7.5 g/100 ml. Serum electrophoresis nor-

mal. Total lipoids (Kunkel) 650 mg/100 ml. Serum cholesterol 176–222 mg/100 ml. Alkaline phosphatases 12 Beassey-Lowry units (normal for this age up to 13 IU/L units). NPN 26 mg/100 ml.

Glucose tolerance curve normal. PBI 8 µg/100 ml. Triiodothyronine uptake in erythrocytes 14%. BMR -7 to +27.

Urinary excretion of 5 HIAA 9 mg/24 h. GOT 30 units. GPT 58 units. Total gonadotropins normal (>5 <20 MU/24 h).

Urinary excretion of 17 ketosteroids 3.0–7.0 mg/24 h. 17 ketogenic steroids 5.4–23 mg/24 h. Normal response to the metopirone and dexamethasone tests.

Urine concentration test showed a maximal concentration capacity of 1079 and an osmolality of 1058 mosm, i.e. normal values. Sodium 139–143 mEq/l serum. Potassium 3.4–4.3 mEq/l serum. Chloride 103–108 mEq/l serum. Calcium 10.8–11.4 mg/100 ml serum (5.4–5.7 mEq/l serum). Serum phosphorus 4.4–3.2 mg/100 ml.

Intravenous infusion of 15 mg calcium per kg body weight during four hours did not change the level of the serum phosphorus values (4.5–4.7 mg/100 ml).

Urinary calcium 80–170 mg/24 h.

Normal cerebrospinal fluid.

#### Other examinations

Roentgenological examination of the skull showed normal conditions with a sella turcica of normal size and shape. Cerebral pneumoencephalogram and X-rays of the heart and lungs were normal. Roentgenography of the skeleton showed a normal bone age of 13 years and open epiphyseal lines, no osteoporosis. On retroperitoneal air insufflation followed by abdominal aortography, enlargement of the left adrenal gland could not be excluded. In view of the possibility of a tumour this gland was explored and removed, however it was found to be of normal size (weight 7.4 g) and histological examination showed normal adrenal gland tissue.

A Mantoux test gave negative results. EEG pathological with episodes of abnormality and no distinct foci. ECG normal with no shortening of the QT distance. Biopsy of the testes showed in adult picture with fully developed spermatocytes.

#### Further course and treatment

Because of a successive increase in the serum calcium values (to a maximum of 11.4 mg/100 ml serum or 5.7 mEq/l) the parathyroid glands were explored and a parathyroid adenoma, the size of a small gooseberry, was found and removed. Histological examination of the adenoma (Fig. 2) showed parathyroid tissue with interspersed fat lobules and mainly built up of chief cells in solid accumulations or alveolar structures.

After the operation the headaches disappeared and the patient's general condition improved. The serum calcium values decreased to 9.9–10.6 mg/100 ml and the phosphorus values were 4.8–3.5 mg/100 ml. There were no postoperative complications and no



Fig 2 Parathyroid adenoma composed mainly of chief cells

signs of hypocalcaemia. Probably due to the natural occurrence of puberty the boy increased in height and his obesity became less pronounced. His achievement at school and his general ability appeared normal during two years of observation after the operation.

#### *Summary and discussion*

The case presented here offered considerable diagnostic difficulties. The onset with increasing obesity and diffuse headache in association with the death of the boy's mother would appear to indicate a psychogenic origin of the symptoms and a psychogenic element in the picture cannot be excluded.

The presence of an adrenal tumour as a reason for the boy's shortness of stature and obesity could not be excluded and exploration and removal of the left adrenal gland were therefore performed but normal conditions were found. At first the slightly elevated blood calcium values aroused a suspicion of sarcoidosis which diagnosis was not supported however either by the rest of the laboratory findings, roentgenological examinations or liver biopsy.

Preoperatively there appeared to be no

strong reasons to consider a diagnosis of hyperparathyroidism. Apart from the elevated blood calcium values and unchanged phosphorus levels during calcium infusion there were no laboratory data to support the diagnosis and no classical symptoms or pathological roentgenographic findings. Nevertheless the hypercalcaemia and the unclear picture in other respects were considered sufficient to motivate exploration of the parathyroid glands which resulted in the final diagnosis after the removal of a parathyroid adenoma the general condition of the patient improved, his headaches disappeared and the blood calcium values returned to normal.

#### DISCUSSION

On studying the literature we found reports of altogether 42 children with primary hyperparathyroidism the first being from the year 1930 (see Table 1). Including our own case the cases reviewed here consists of 43 children (18 girls and 25 boys) of ages 0-15 years. (A number of further patients were reported to have hyperparathyroidism (3, 10, 13, 25 and



Table 1 Primary hyperparathyroidism in children: a summary of case reports from the literature

Publication Name	Year	Sex			Age in years	Symptoms and signs	Serum Ca <sup>2+</sup> mg/100 ml	Serum phosphorus mg/100 ml	Urinary Ca <sup>2+</sup> mg/24 hrs	Findings at X-ray examination	Duration of symptoms (yr)	Autopsy	Operation	Histology	
		No	M	F										Hyper- plasia	Adenoma
Philips (34)	1948	1			4/12	Vomiting, dehydration	—	—	—	Extensive osteoporosis	4/12	+		+	Unknown
Randall & Lauchlan (38)	1963	1			9/12	Anorexia, emaciation	20	2.3	—	Gen osteoporosis	9/12	+		+	
Pruitt <i>et al</i> (35)	1947	3			10/12	Weakness, constipation, vomiting	22.1	3	—	Undercalcification irregularity of cortex	10/12	+		+	
Fretwell & Gardberg (17)	1963	4	×		1	Constipation, mental retardation, anorexia	24	2.3	—	Gen osteoporosis	1		+	+	
Rogier & Beau- doin (42)	1947	5	×		2½	Vomiting, constipation, genus recurv	15	3.4	—	Gen osteoporosis, resorption of lamina dura, renal calculi	2		+	+	
Crawford <i>et al</i> (11)	1956	6			3	Lump, tubercle, face	14	2.1	—	Osteoporosis, cyst	1		+	+	
Lee <i>et al</i> (26)	1955	7	×		7	Vomiting, blindness B.P. 180/130	20	2.5	—	Osteoporosis, renal lithiasis absent	1		+	+	
Hamrick <i>et al</i> (18)	1964	8	×		7	Depression, weakness	12	2.8	—	Fracture, cyst	1			+	
Pugh (36)	1946	9	×		8	Polydipsia, headache, vomiting	19	2.8	—	Renal lithiasis, osteoporosis rare	8/12	+	+	+	
Jones (23)	1955	10	×		9	Polydipsia, constipation, vomiting	19	—	600	No renal lithiasis	2		+	+	
Tennison <i>et al</i> (48)	1955	11	×		10	Fracture, vomiting	17	2.7	—	Renal lithiasis, duodenal ulcer	1		+	+	
Nolan <i>et al</i> (31)	1960	12		×	11	Abdominal pain	13	2.8	300	Normal roentgen	2		+	+	
Wood <i>et al</i> (50)	1958	13			12	Alopecia, flaking of nails	17.5	2.1	—	Subperiosteal erosions	1½		+	+	
Feitz (16)	1961	14			12	Polyuria, hypernatremia, vomiting	17	—	360	Normal right carotid angiography	1/12		+	+	
Senus <i>et al</i> (44)	1963	15		×	12	Hemiparesis, vomiting, muscle weakness	15	2.5	—	Subperiosteal resorp- tion, osteoporosis	2½		+	+	
Churchill & Cope (9)	1934	16	×		13	Thirst, enuresis, fracture, bone pain	12	4.7	—	Stump renal stone, cysts decalcification	4		+	+	
Albright <i>et al</i> (2)	1934	17		×	13	Polyuria, fractures, weakness, convulsions	16.6	2.2	—	Osteoporosis	4		+	+	
Atsuzaki & Clifton (3)	1939	18			13	Renal stones, nephro- lith, bradycardia	15.5	2.8	600	Renal lithiasis, osteoporosis	1		+	+	
Beckel (5)	1945	19	×		13	Polyuria, uremia, gonorr- hoea	—	—	—	No skeletal changes	—	+		+	
Arli & Asang (4)	1953	20	×		13	Arthralgia, gonorr- hoea	13.9	3.0	—	Renal lithiasis	—		+	+	
Wondoloff <i>et al</i> (6)	1954	20	×		13	Arthralgia, gonorr- hoea	—	—	—	Renal lithiasis	—		+	+	





and minimal serum phosphorus levels in the 38 children of the series for whom these values were reported (see Table 1). Twenty eight of these 38 children had serum calcium values of 15 mg/100 ml or higher. Among the 6 patients who were reported later than 1965 (see Table 1) the maximal serum calcium value was 15.4 mg/100 ml.

The calcium excretion in the urine had been measured in 17 patients and in 12 of them the urinary calcium per 24 hours exceeded 400 mg.

Renal function tests (NPN, serum creatinine and maximal specific gravity of urine) were reported for 23 children. In 11 of these the findings indicated unimpaired function but in no case were there signs of severe renal damage.

As can be seen in Tables 1 and 3 35 children (18 boys and 17 girls) had one adenoma. In case 29 there were two adenomas and in case 39 the parathyroid adenoma was located within the thymus gland. Seven patients had hyperplasia and in one of them (case 3) chief cell hyperplasia was noted. All 7 patients with hyperplasia were boys and 4 of them were less

Table 3 Classification of 43 children with primary hyperparathyroidism according to the findings at histological examination of the parathyroid glands and determinations of the serum calcium level

Parathyroid glands	Cases		Mean age (years)	Serum calcium >17 mg/100 ml	
	Total no	Boys ( )		No	%
Adenoma	35	31	12.8	13 (out of 37)	41
Hyperplasia	7	100	3.2	5 (out of 6)	83
Unknown	1	0	13	1	0

than 2 years old (see Table 1). The serum calcium values were much higher throughout in the patients with hyperplasia than in those with adenoma (see Table 3).

No case of parathyroid carcinoma was found in the literature reviewed.

In adults primary hyperparathyroidism seems to be more common among women than men. Norris (32) reviewed 336 cases from the literature and 60% of them were women. Among the 138 patients with hyperparathyroidism reported by Hellstrom & Ivarmark (20) there were 99 women (72%). Similarly in another Swedish series (47) of 85 cases 69 were women. Thus the ratio of women to men with hyperparathyroidism in adult series seems to be about 3:2 while the corresponding ratio in this paediatric series is 2:3. This finding which demonstrates that hyperparathyroidism is more common among boys than among girls agrees with the observation of Hellstrom & Ivarmark (20) that only 58 (19 out of 33) of the patients below 40 years of age were women.

Hyperplasia of the parathyroids seems to be relatively more common in children than in adults. This condition was found in 7 (19%) of the 43 patients presented above while with hyperparathyroidism found only 8.5% with hyperplasia. Hellstrom & Ivarmark (20) reported that 17 (12%) of their 138 patients showed hyperplasia and Thoren & Werner

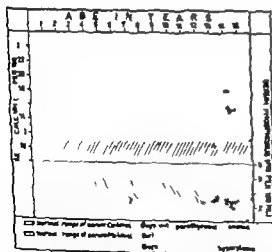


Fig. 3 Maximal serum calcium and minimal serum phosphorus levels in 38 children with primary hyperparathyroidism (in 5 out of 43 cases no values of serum calcium or phosphorus were given, see Table 1).

Table 2 Incidence of main symptoms signs and X ray findings in the series of 43 children with primary hyperparathyroidism

In 2 children (cases 29 and 37) there were no reports of X ray findings and in one of them (case 29) there was also no report of the symptoms and signs see Table 1

Symptoms and signs	No of cases according to age																No of cases			
	Age in years																M	F	Total	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				16
Fatigue weakness anorexia																				
constipation vomiting		3	1	1					3	1	1	1		2	4	5	2	15	9	24
Nervousness irritability																				
depression headache		1							1	1				1		1		3	2	5
Mental retardation infantism																				
blindness dysarthria diplopia			1					1					1	2	1	1		6	1	7
Alopecia												1						1	1	
Gastroduodenal ulcers											1				1 <sup>9</sup>			2	0	2
Polyuria & polydipsia										1	1			2	4	3	1	6	6	12
Hypertension								1					1		2	2		4	2	6
Bone & joint symptoms fracture				1	1							1		3	6	4		8	10	16
X ray findings																				
Skeletal changes		3	1	1	1				2	1				2	6	7	5	17	17	34
Renal lithiasis				1					1			1			3	3	1	7	4	11
																+17				
Renal lithiasis and skeletal changes				1					1					3	1			5	1	6

43) but the diagnosis in these cases is considered uncertain and they are therefore not included in our study)

A review of the 43 patients is presented in Table 1

Table 2 shows the incidence of the main symptoms and X ray findings in the 43 patients

The predominating symptoms were weakness anorexia and irritability which were present in more than 50% of the patients. Constipation was noted in 7 children and polyuria and polydipsia in 12. Local skeletal changes were observed in 21 and renal calculi in 11 of the 43 patients.

Complete heart block was noted in case 7 (Table 1) while in no other case was shortening of the Q-T interval observed.

Three patients had had parathyroid crises (Table 1 cases 7, 14 and 21) their serum calcium levels lay at 20, 17 and 22 mg/100 ml respectively. Residual cerebral and renal lesions were noted in case 7 probably due to the long duration of the disease before operation. In case 14 there was immediate and complete improvement after the operation both clinically and as regards the laboratory

findings. The postoperative improvement was also satisfactory in case 21.

EEG recordings were made in 7 children. The tracings were clearly pathological in case 14 with parathyroid crises which on two occasions led to coma and temporary hemiparesis seven months after the operation however a distinct improvement of the EEG pattern was noted. This patient was described as a case of hypertensive encephalopathy and had a blood pressure of 180/110 mm Hg during the crises. In the youngest patient with parathyroid crises (case 7) on the other hand the EEG tracings showed deterioration after operation with a diffusely irregular pattern and the boy became blind. No EEG recordings were made in the oldest patient with parathyroid crises (case 21). In one child (case 40) the EEG pattern indicated a non specific dysfunction in the upper part of the brain stem. In our patient (case 43) there were minor abnormalities such as varying episodes with no distinct foci and polymorphic episodes over the temporal lobes. In three patients (cases 3, 4 and 32) the EEG tracings showed nothing abnormal.

Fig. 3 shows the maximal serum calcium

and minimal serum phosphorus levels in the 38 children of the series for whom these values were reported (see Table 1). Twenty eight of these 38 children had serum calcium values of 15 mg/100 ml or higher. Among the 6 patients who were reported later than 1965 (see Table 1) the maximal serum calcium value was 15.4 mg/100 ml.

The calcium excretion in the urine had been measured in 17 patients and in 12 of them the urinary calcium per 24 hours exceeded 400 mg.

Renal function tests (NPN serum creatinine and maximal specific gravity of urine) were reported for 23 children. In 11 of these the findings indicated impaired function but in no case were there signs of severe renal damage.

As can be seen in Tables 1 and 3 35 children (18 boys and 17 girls) had one adenoma. In case 29 there were two adenomas and in case 39 the parathyroid adenoma was located within the thyroid gland. Seven patients had hyperplasia and in one of them (case 3) chief cell hyperplasia was noted. All 7 patients with hyperplasia were boys and 4 of them were less

Table 3 Classification of 43 children with primary hyperparathyroidism according to the findings at histological examination of the parathyroid glands and determinations of the serum calcium level

Parathyroid glands	Cases		Mean age (years)	Serum calcium > 17 mg/100 ml	
	Total no	Boys ( )		Yes	No
Adenoma	35	51	12.8	13 (out of 32)	41
Hyperplasia	7	100	3.2	5 (out of 6)	83
Unknown	1	0	13	0	0

than 2 years old (see Table 1). The serum calcium values were much higher throughout in the patients with hyperplasia than in those with adenoma (see Table 3).

No case of parathyroid carcinoma was found in the literature reviewed.

In adults primary hyperparathyroidism seems to be more common among women than men. Norris (32) reviewed 336 cases from the literature and 60.4% of them were women. Among the 138 patients with hyperparathyroidism reported by Hellstrom & Ivarmark (20) there were 99 women (72%). Similarly in another Swedish series (47) of 85 cases 69% were women. Thus the ratio of women to men with hyperparathyroidism in adult series seems to be about 3:1 while the corresponding ratio in this paediatric series is 2:3. This finding which demonstrates that hyperparathyroidism is more common among boys than among girls agrees with the observation of Hellstrom & Ivarmark (20) that only 33% (19 out of 57) of the patients below 40 years of age were women.

Hyperplasia of the parathyroids seems to be relatively more common in children than in adults. This condition was found in 7 (19%) of the 43 patients presented above while with hyperparathyroidism found only 8.5% with hyperplasia. Hellstrom & Ivarmark (20) reported that 17 (12%) of their 138 patients showed hyperplasia and Thoren & Werner

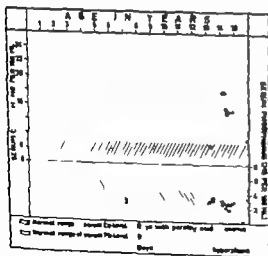


Fig 3 Maximal serum calcium and minimal serum phosphorus levels in 38 children with primary hyperparathyroidism (in 5 out of 43 cases no values of serum calcium or phosphorus were given see Table 1).

(47) gave a figure of 5 (7%) out of 85. All 7 patients with cellular hyperplasia in the present series of 43 children were boys, and mainly from the lowest age groups.

The renal lesions seem to be less pronounced in children than in adults and the incidence of renal calculi lower reasonably due to a shorter duration of the disease in children. Only 11 (25%) of the 43 paediatric patients had renal calculi which may be compared with 86% of the adult series of Hellstrom & Ivarmark (20) and 59% of the adult series of Thorén & Werner (47).

Primary hyperparathyroidism has been said to give a higher incidence of skeletal changes and a higher degree of hypercalcaemia in children than in adults. Thus local skeletal changes were reported in 20 of the 43 paediatric patients presented here while Thorén & Werner (47) observed such changes in only 9 out of 85 adult patients.

Thorén & Werner (47) conclude that the increase in the number of diagnosed adult cases of primary hyperparathyroidism noted in recent years mainly concerns patients with renal calculi and atypical symptoms (such as fatigue, weakness, muscular atrophy and constipation) while the number of cases with skeletal involvement seems to remain essentially unchanged.

The clinical picture of primary hyperparathyroidism is often non-specific and inconspicuous. The diagnosis thus has to be based upon laboratory findings, mainly the serum calcium level. This level sometimes can be only moderately elevated as is illustrated by the cases presented above. It may perhaps be significant that the maximal serum calcium level was not higher than 15.4 mg/100 ml in any of the 6 cases diagnosed later than in 1965.

In order to avoid severe and sometimes irreparable damage as a result of hyperparathyroidism an early diagnosis seems to be essential and the serum calcium level ought to be measured in children with unexplained symptoms such as anorexia, weakness, constipation and increased thirst.

## SUMMARY

A case of primary hyperparathyroidism in a 13 year old boy is reported showing some difficulties in diagnosing this disorder and pointing out that the disease may occur with only minor increases in the serum calcium values.

Forty-two children with hyperparathyroidism have been found in the literature, and some data for these patients are also presented and discussed.

In children primary hyperparathyroidism is more common among males than females (ratio 3:2) while in adults this sex relationship is reversed (ratio 2:3). In about one-fifth of the children (all boys) the condition was due to diffuse enlargement of the parathyroids; these boys also showed higher serum calcium levels than the patients with adenoma. In adults diffuse hyperplasia occurs in only one-tenth of the patients.

Primary hyperparathyroidism seems to be accompanied by a higher incidence of skeletal changes and, as a rule, a higher degree of hypercalcaemia in children than in adults, while renal lesions seem to be less pronounced and the incidence of renal calculi lower.

General symptoms of weakness, anorexia, muscular atrophy and constipation as well as increased thirst in children should arouse suspicion of hyperparathyroidism and the serum calcium level should be determined so that an early diagnosis may be made and treatment given.

## REFERENCES

1. Adam A. & Ritchie M. Hyperparathyroidism with increased bone density in the areas of growth. *J Bone Joint Surg (Amer)* 36 B 257 1954.
2. Albright F, Aub J C & Bruer W. Hyperparathyroidism: a common and polymorphic condition as illustrated by seventeen proved cases from one clinic. *JAMA* 102 9 1934.
3. Anspach W E & Clifton W M. Hyperparathyroidism in children: report of two cases. *Amer J Dis Child* 59 540 1939.
4. Arlt H G & Aaron H. Intestinal Hyperparathyroidismus. *Z Kinderheilk* 73 294 1953.
5. Bickel G. Démonstrations cliniques. *Helv Med Acta* 12 273 1945.

6. Bordasoff M D, Woods A A, White J E & Engel F L. Hyperparathyroidism. *Amer J Med* 1 538 1956
7. Brown I. Primary hyperparathyroidism med neurologiska symtom. Et tillfælde hos en 14-årig dreng. *Ugeskr Læge* 127/13 1964
8. Charvet-Carballo R & Hayles A B. Parathyroid adenoma in children. Report of three cases with unusual articular manifestations in one case. *Amer J Dis Child* 112 553 1966
9. Churchill E D & Cope O. Parathyroid tumors associated with hyperparathyroidism. *Surg Gynec Obstet* 58 255 1934
10. Cooley T B. Hyperparathyroidism and similar diseases of bone. *Trans Amer Pediatr Soc* 43 70 1931
11. Crawford H J M, Sefton J & Alvarez A F. Three unusual cases of hyperparathyroidism. *Brit J Surg* 44 193 1956
12. Drieser R. Osteitis fibrosa cystica associated with parathyroid overactivity. *Amer J Roentgen* 30 596 1933
13. Dörken J. Beitrag zur Kenntnis der endokrinen Erkrankungen des kindlichen Skelettsystems. *Z Kinderheilk* 46 114 1928
14. Dyle S C, Walker R M & Freeman J. Adenoma of the parathyroid associated with generalized osteitis fibrosa. *Lancet* ii 530 1933
15. Fahrén H S. Parathyroid tumors: report of 2 new cases and discussion of these and 5 cases previously reported. *Trans Amer Gynecol Ass* 232 1240
16. Fenzl V. Hyperkalcæmie encephalopathy in a child. *Acta Neurol Scand* 37 307 1963
17. Fretchen B & Gurdberg O. Primary hyperparathyroidism in an infant. *Acta Chir Scand* 179 557 1963
18. Hanclick M, Jelonek A, Mostala D & Badowski Z. Primary hyperparathyroidism in a 7-year-old boy. *Endokr Pol* 13 27 1964
19. Harrison M. Parathyroid adenoma in a child. *Amer J Dis Child* 91 513 1956
20. Hellstrom J & Jonasson B I. Primary hyperparathyroidism: clinical and structural findings in 134 cases. *Acta Chir Scand* 294 1962
21. Hocco D, Border Ph, Mazzaboni A, De Sèze S & Lichtwitz A. Cas observations d'adénomes parathyroïdiens adénocarcinomes. Confrontations cliniques radiologiques anatomiques et biochimiques. *Presse Med* 69/1 153 1961
22. Jonasson B I. Perinatal communication 1966
23. Jorren J H I. Een Geval van Hyperparathyroïdisme bij de kinderteit. *Nederl T Geneesk* 99 1 690 1955
24. Leonard D W, Meuser J E & Bryan J E. Hyperparathyroidism due to an intrathyroid parathyroid adenoma. *Southern Med J (Birmingham Ala)* 55 870 1962
25. Landon J F. Parathyroidectomy in generalized osteitis fibrosa cystica. *J Pediatr* 1 544 1932
26. Lee C M, McElhenny W T & Goff E A. Unusual manifestations of parathyroid adenoma. *Arch Surg (Chicago)* 71 475 1955
27. Lloyd H M, Aitken R E & Ferner T M. Primary hyperparathyroidism resembling rickets of late onset. *Brit Med J* ii 853 1965
28. Lomax, E, Sepulveda L & Stevenson C. Primary hyperparathyroidism simulating rickets. *J Clin Endocr* 26 309 1966
29. McClure R D & Lam C R. End results in the treatment of hyperparathyroidism. *Ann Surg* 121 454 1945
30. Myers N A. Parathyroid adenoma. *Med J Aust* 50/1 735 1963
31. Nolan R B, Hayles A B & Woolner L B. Adenoma of the parathyroid gland in children. Report of case and brief review of the literature. *Amer J Dis Child* 99 622 1960
32. Norris E H. The parathyroid adenoma. A study of 322 cases. *Surg Gynec Obstet* 84 1 1947
33. Penherson J de J & Gaidhe J B. Hyperparathyroidism. *Ann Surg* 9 20\* 1930
34. Phelps R N. Primary diffuse parathyroid hyperplasia in an infant of four months. *Pediatr* 129 429 1948
35. Pratt E L, Owen B B & Neubauer E B II. Hypercalcemia and idiopathic hyperplasia of the parathyroid glands in an infant. *J Pediatr* 30 389 1947
36. Pugh R J. Primary hyperplasia of the parathyroids in a boy aged 8 years. *Proc Roy Soc Med* 39 694 1945-1946
37. Race G J. Primary hyperparathyroidism masked as primary renal disease with hypertension. *N C Med J* 15 659 1952
38. Rapraeger L, Purn O A, Ratnanke V I & de Forreka C P. Parathyroid adenoma in childhood. *Am J Dis Child* 107 442 1964
39. Randall C & Loochian S C. Parathyroid hyperplasia in an infant. *Am J Dis Child* 105 364 1963
40. Remfrank R F & Edwards T L. Parathyroid crisis in a child. *J Am Med Ass* 178 11 468 1961
41. Reimhoff W F Jr. The surgical treatment of hyperparathyroidism with a report of 27 cases. *Ann Surg* 131 917 1950
42. Roger J & Beaumont A. Un cas d'hyperparathyroïdisme primitif chez un enfant de 30 mois. *Pediatric* 14 21 1947
43. Sano T, Hirooka M, Hayashi Y, Iseki E & Tanabe S. Primary hyperparathyroidism in childhood: a case report. *Ann Pediatr Fenn* 4 14 1958
44. Senas M S, Bilbo R E & Smith D W. Parathyroid adenoma in a child. *J Pediatr* 62 876 1963
45. Sèze S de, Lichtwitz A, Hocco D, Border Ph & Parlier R. Tests biochimiques d'exploration de la fonction parathyroïdienne dans les hyperparathyroïdismes primitifs. *Rev Rhum* 28 483 1961
46. Shallow T A & Fyfe E. Parathyroid ade-



- noma occurrence in father and daughter *Surgery* 24 1020 1948
- 47 Thoren L & Werner I Hyperparathyroidism clinical observations in a series of 85 patients *Acta Chir Scand* 135 395 1969
- 48 Tatum H Jensen E Hunnicutt A J Foreman N & Kinsell L W Juvenile hyperparathyroidism in association with peptic ulcer *J Clin Endocr* 15 1141 1955
- 49 Wilkins L *The diagnosis and treatment of endocrine disorders in childhood and adolescence* Blackwell Scientific Publications Oxford 1957 2nd edition
- 50 Wood B S B George W H & Robinson A W Parathyroid adenoma in a child presenting as rickets *Arch Dis Child* 33 46 1958
- 51 Woolner L B Keating F R & Black B M Tumors and hyperplasia of the parathyroid glands *Cancer* 5 1069 1952

Submitted Jan 17 1969

Accepted Oct 28 1969

(I W) Dept of Medicine

Akademiska Sjukhuset

750 14 Uppsala

Sweden

Key words Hyperparathyroidism children review

## THE INFLUENCE OF ADENINE ON THE CLINICAL FEATURES AND PURINE METABOLISM IN THE LESCH NYHAN SYNDROME

J. P. M. VAN DER ZEE, E. J. P. LOVMEIJN, J. M. F. TRUBELS  
and E. H. A. M. SCHREUTEN

From the Paediatric Department University of Nijmegen Nijmegen the Netherlands

The Lesch Nyhan syndrome is characterized by mental retardation choreoathetosis aggressive behaviour with autostimulation and hyperuricaemia (9, 17, 18). A number of authors have mentioned the occurrence of a megaloblastic anaemia in this syndrome (7, 11, 12, 18, 24). The syndrome is an X-linked recessive disorder (13, 16, 23). The purine synthesis *de novo* is greatly increased in these patients (9, 15). Seegmiller found a deficiency of the enzyme hypoxanthine guanine phosphoribosyl transferase in patients with this syndrome (22). The relationship between the greatly increased purine synthesis *de novo* and this enzyme deficiency is at present an important feature of investigation (20).

We studied the influence of orally administered adenine on the clinical features (25) and on the purine metabolism of two patients with the Lesch Nyhan syndrome.

### METHODS

During the time of the investigation two patients with this syndrome, Re W and Ru W (Fig. 1 no 3 and 5) were given a purine free diet. The uric acid was determined according to the enzymatic spectrophotometric method (10). The glycine- $^3\text{H}$  was orally administered in a dose of 5  $\mu\text{Ci}$  to Re W and 4  $\mu\text{Ci}$  to Ru W. For the first two days after administration of glycine- $^3\text{H}$  urine was collected in 12-hour periods, thereafter in 24-hour periods. The uric acid was isolated from three samples, purified and the  $^3\text{H}$  activity of uric acid was measured according to the method of Lesch & Nyhan (9). The activity of

the enzyme hypoxanthine guanine phosphoribosyl transferase was measured according to the method of Carter (2).

### RESULTS

We have already described the symptoms of the boys in family W: H W (no 1), Re W (no 3) and Ru W (no 5) (Fig. 1) (24). The most important clinical data and laboratory results obtained from Re W and Ru W are shown in Table 1. The results of the investigation of the purine metabolism in these patients are shown in Tables 2 and 3. We found no activity of the enzyme hypoxanthine guanine phosphoribosyltransferase in the erythrocytes of Re W and Ru W (Table 4). The haematological findings are shown in Table 5.

#### Bone marrow Re W

The bone marrow showed a normal ratio of erythropoiesis and leucopoiesis. As well as a normoblastic there was also a megaloblastic maturing sequence. In the megaloblasts a regularly divided chromatin structure was visible. The erythropoiesis showed an inhibition of nuclear division (Fig. 3). In the white sequence macrocytocytes were also present.

#### Bone marrow Ru W

The bone marrow of this youngest patient showed a normal ratio of erythropoiesis and leucopoiesis. The red sequence seemed to be normal. There were no convincing megalob-

- noma occurrence in father and daughter *Surgery* 24 1020 1948
- 47 Thoren L & Werner I Hyperparathyroidism clinical observations in a series of 85 patients *Acta Chir Scand* 135 395 1969
- 48 Tsumori H, Jensen E, Hunnicutt A J, Foreman N & Kinsell L W Juvenile hyperparathyroidism in association with peptic ulcer *J Clin Endocr* 15 1141 1955
- 49 Wilkins L *The diagnosis and treatment of endocrine disorders in childhood and adolescence* Blackwell Scientific Publications Oxford 1957 2nd edition
- 50 Wood B N B, George W H & Robinson A W Parathyroid adenoma in a child presenting as rickets *Arch Dis Child* 33 46 1958
- 51 Woolner L B, Keating F R & Black B M Tumors and hyperplasia of the parathyroid glands *Cancer* 5 1069 1952

Submitted Jan 17 1969

Accepted Oct 28 1969

(I W) Dept of Medicine

Akademiska Sjukhuset

750 14 Uppsala

Sweden

**Key words** Hyperparathyroidism children review

## THE INFLUENCE OF ADENINE ON THE CLINICAL FEATURES AND PURINE METABOLISM IN THE LESCH NYHAN SYNDROME

S. P. M. VAN DER ZEE, E. J. P. LOMMEN, J. M. F. TRUBELS  
and R. D. A. M. SCHRETTEN

*From the Paediatric Department University of Nijmegen Nijmegen the Netherlands*

The Lesch Nyhan syndrome is characterized by mental retardation choreoathetosis aggressive behaviour with automutilation and hyperuricaemia (9 17 18). A number of authors have mentioned the occurrence of a megaloblastic anaemia in this syndrome (7 11 12 18 24). The syndrome is an X-linked recessive disorder (13 16 23). The purine synthesis *de novo* is greatly increased in these patients (9 15). Seggiller found a deficiency of the enzyme hypoxanthine guanine phosphoribosyl transferase in patients with this syndrome (22). The relationship between the greatly increased purine synthesis *de novo* and this enzyme deficiency is at present an important feature of investigation (20).

We studied the influence of orally administered adenine on the clinical features (25) and on the purine metabolism of two patients with the Lesch Nyhan syndrome.

### METHODS

During the time of the investigation two patients with this syndrome Re W and Ru W (Fig 1 no 3 and 5) were given a purine free diet. The uric acid was determined according to the enzymatic spectrophotometric method (10). The glycine-2-<sup>14</sup>C was orally administered in a dose of 5  $\mu$ C to Re W and 4  $\mu$ C to Ru W. For the first two days after administration of glycine-2-<sup>14</sup>C urine was collected in 12 hour periods thereafter in 24 hour periods. The uric acid was obtained from these samples purified and the C activity of uric acid was measured according to the method of Lesch & Nyhan (9). The activity of

the enzyme hypoxanthine guanine phosphoribosyl transferase was measured according to the method of Carter (2).

### RESULTS

We have already described the symptoms of the boys in family W: H W (no 1) Re W (no 3) and Ru W (no 5) (Fig 1) (24). The most important clinical data and laboratory results obtained from Re W and Ru W are shown in Table 1. The results of the investigation of the purine metabolism in these patients are shown in Tables 2 and 3. We found no activity of the enzyme hypoxanthine guanine phosphoribosyltransferase in the erythrocytes of Re W and Ru W (Table 4). The haematological findings are shown in Table 5.

#### *Bone marrow Re W*

The bone marrow showed a normal ratio of erythropoiesis and leucopoiesis. As well as a normoblastic there was also a megaloblastic maturing sequence. In the megaloblasts a regularly divided chromatin structure was visible. The erythropoiesis showed an inhibition of nuclear division (Fig 3). In the white sequence macrophages were also present.

#### *Bone marrow Ru W*

The bone marrow of this youngest patient showed a normal ratio of erythropoiesis and leucopoiesis. The red sequence seemed to be normal. There were no convincing megalob-

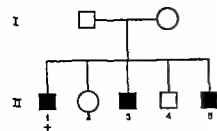


Fig 1 Family of our patients

lasts however, the young cells showed finely divided chromatin structure. The leucopoiesis seemed normal, the number of megakaryocytes was normal.

None of the patients received anticonvulsive therapy in the form of diphenhydramine or mephobarbital.

Table 1 The most important clinical data and laboratory results in patients Re W and Ru W

	Re W	Ru W
Date of birth	3 8 1959	14 3 1966
Age at the time of the investigation	9 years	2 years
Mental retardation	+	+
Choreoathetosis	+	+
Autism	+	+
Serum urea mg/100 ml	48-59	41-44
Serum creatinine mg/l	6.1-7.1	4.4-4.7
Urine protein	Negative	Negative
sediment	Uric acid	Uric acid
	Crystals	Crystals
cultures	Negative	Negative
Urea clearance ml/min/1.73 m <sup>2</sup>	21.9-24.9	28.0-41.0
Endogenous creatinine clearance ml/min/1.73 m <sup>2</sup>	45.0-59.7	45.2-59.2
Concentration ability	sw. 1013 osmol 364	sw. 1022 osmol 640
Phenolred excretion after 2 hours	65	60
Phosphate-clearance in of the creatinine-clearance	57-77	86-89
I V pyelogram	No anatomical aberration	No anatomical aberration
Glucosuria	Negative	Negative
Acid load with 100 mEq NH <sub>4</sub> Cl per m <sup>2</sup> per 24 hours expressed in indices according to Elkington (15)		
Norm		
T A 3rd day 0.6-1	0.34	0.45
5th day 0.4-1	0.21	
NH <sub>4</sub> 3rd day 0.8-2.4	0.74	0.90
5th day 0.9-2.7	0.80	
H 3rd day 1.4-3.4	1.0	1.36
5th day 1.5-3.4	0.85	

Table 2 The serum uric acid concentrations and urinary uric acid excretions in patients Re W and Ru W

	Re W	Ru W	Controls
Serum uric acid mg/100 ml	8.6-11.7	8.9-10.3	3.0-4.5
Uric acid excretion in urine mg/24 hours	642-714	456-600	176-787*
Uric acid excretion mg/kg/24 hours	43.4-48.2	42.2-55.5	10.3*
Excretion ratio uric acid/creatinine/24 hours	1.6-2.4	1.8-3.1	0.66*

\* Published data from Nyhan (2)

\* Published data from Kaufman et al (17)

Table 3 The cumulative excretion of isotope in urinary uric acid after oral gift of glycine <sup>14</sup>C in patients Re W and Ru W

	Re W	Ru W	Controls
7 days recovery of isotope in urinary uric acid	2.48	1.63	0.15

\* Published data from Nyhan who administered the glycine <sup>14</sup>C intravenously

Table 4 Activity of adenine and hypoxanthine guanine phosphoribosyltransferase in haemolysates of erythrocytes of controls and patients Re W and Ru W

	Adenine and hypoxanthine guanine-phosphoribosyltransferase activity in mpmol/ml red cells/min		
	Substrate adenine	Substrate guanine	Substrate hypoxanthine
Controls (mean value $\pm$ 1 s.d.)	134 $\pm$ 42	710 $\pm$ 121	323 $\pm$ 91
Patient Re W	180	Non detectable	Non detectable
Patient Ru W	241	Non detectable	Non detectable

The oldest boy in this family H W who died at two and half year age had also a megaloblastic anaemia. Both patients H W and Re W showed no improvement in their megaloblastic anaemia after intramuscular injections

Table 5 Haematological findings in patients Re W and Ru H

	Re W	Ru W
Age at the time of investigation	9 years	2 years
Hb g/100 ml	8.4-9.0	11.6-13.1
Erythrocytes $\mu\text{m}^3$ count	1.64-1.83	3.36-3.53
Hematocrit %	25-27	36.3-39.5
Reticulocytes %	7-14	15-14
MCH $\mu\text{g}$	33.5-33	31.5-33
MCV $\mu\text{m}^3$	153-147	108-112
MCHC g/g	52-49	35-37
Leucocytes $\mu\text{m}^3$ count	9 400-10 700	7 000-10 700
Thrombocytes $\mu\text{m}^3$ count	64 000-314 000	184 000-348 000
TIBC g/100 ml	3.6	4.35
Serum iron g/100 ml	61	107
Iron saturation	1	24
Osmotic resistance		
50 haemolysis	0.48 NaCl	0.48 NaCl
Autohaemolysis	Normal	Normal
Survival time of own erythrocytes T <sub>50</sub> Cr	31 days	
Histamine fast achlorhydria	Absent	Absent
Vitamin B <sub>12</sub> (norm. 50-760 pg/ml)	290	300
Folic acid $\mu\text{g}$ ml (norm. 7-30 $\mu\text{g}$ /mg)	6.5	9.6

of 25  $\mu\text{g}$  vitamin B<sub>12</sub> per day and oral administration of 15 mg folic acid per day. Oral administration of adenine to patient Re W resulted in a disappearance of the megaloblastic anaemia (Fig. 2).

Three months after the adenine therapy was terminated the bone marrow once again showed a megaloblastosis (Fig. 3).

We continued to study in this patient and in his brother the effect of the administration of adenine on purine synthesis *de novo* as measured by incorporation of glycine <sup>14</sup>C into urinary uric acid. Indeed we found a diminished incorporation during administration of adenine (Table 6 Figs 4 and 5).

## DISCUSSION

These brothers show the already classical features of the Leish-Nyhan syndrome (9, 17, 18).

The results of our investigation of the hyperuricaemia, the hyperuricuria and the increased glycine <sup>14</sup>C incorporation in urinary uric acid are in close correspondence with those of the other investigators (5, 6, 7, 8, 9, 14, 15, 21, 22).

The kidney function disorders which we diagnosed agree very well with the findings of Marie and Royer and according to them fit in with the picture of a chronic interstitial nephritis (12, 21).

In H W and Re W we found a megaloblastic anaemia refractory to vitamin B<sub>12</sub> or folic acid therapy. In the 3rd patient of the

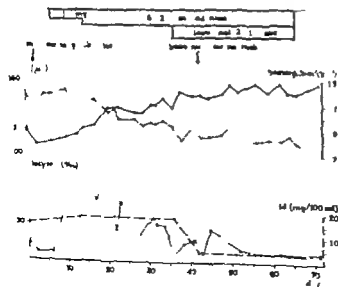


Fig. 2 Response of megaloblastic anaemia to orally administered adenine in patient Re W.

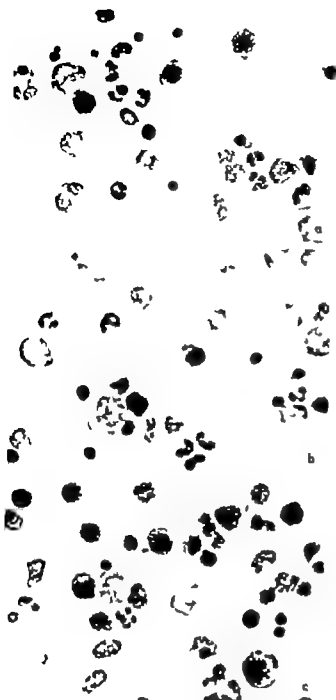


Fig 3 Bone marrow of Re W before *a* during *b* and 3 months after *c* termination of adenine administration

family Ru W the megaloblastosis was less pronounced

On the ground of our investigation and those of other authors (1 7 11 12 18) we believe that a megaloblastic anaemia is a characteristic symptom of the Lesch-Nyhan syndrome. No indication of a vitamin B<sub>12</sub> deficiency was found in any of the patients with megaloblastic anaemia.

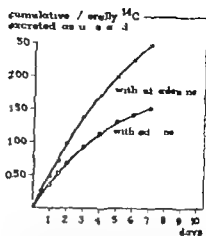


Fig 4 The cumulative excretion of isotope in urinary uric acid after oral gift of glycine-<sup>14</sup>C in patient Re W before and during adenine administration dose adenine 20 mg/kg body weight/day

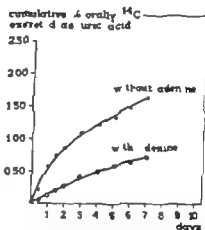


Fig 5 The cumulative excretion of isotope in urinary uric acid after oral gift of glycine-<sup>14</sup>C in patient Ru W before and during adenine administration dose adenine 50 mg/kg body weight/day

Kelley found a decreased folic acid concentration in the serum of his patients (7). Owing to the fact however that in a number of patients with a megaloblastic anaemia no folic acid deficiency existed and our patients never

Table II The cumulative excretion of isotope in urinary uric acid after oral gift of glycine-<sup>14</sup>C before and during adenine administration

	Re W ( )	Ru W ( )
7 days recovery of isotope in urinary uric acid		
without adenine	2.48	1.63
with adenine	1.53	0.72

showed any improvement in their megaloblastic anaemia after folic acid therapy, it cannot be assumed that a folic acid deficiency is the only cause of this megaloblastic anaemia. It is possible that because of the increased purine synthesis *de novo* in Lesch Nyhan patients a greater need for folic acid exists. A justification for this is the discovery by Felix & DeMars that fibroblasts cultures from Lesch Nyhan patients have a strongly increased need for folic acid (4).

It seems probable that the disturbed nucleic acid synthesis in the erythrocytes which is the cause of the megaloblastic anaemia is a result of the unbalanced formation of purine nucleotides.

The disappearance of the megaloblastic anaemia by adenine therapy suggests that in this way the nucleic acid synthesis in the erythrocytes had been improved. In the course of our investigations Felix & DeMars (4) published their findings that fibroblasts cultures from Lesch Nyhan patients did not grow in media to which no adenine or folic acid was added. They also assumed that the increase in cell growth after addition of adenine is a result of an improved nucleic acid synthesis.

During oral medication with adenine we noticed that the glycine  $^{14}$ C incorporation in the urinary uric acid decreased. This effect was not caused by the possible decrease of the kidney function as a result of the adenine administration. The endogenous creatinine clearance and the concentrating ability are not changed under adenine treatment.

The relationship between the deficiency of the enzyme hypoxanthine guanine phosphoribosyltransferase and the increased purine synthesis *de novo* could be found in a decreased feedback inhibition of the purine nucleotides as a consequence of the decreased resynthesis of these nucleotides. On the other hand this relation could also be found in a greater availability of phosphoribosylpyrophosphate (PRPP) as a consequence of the enzyme deficiency which would result in an increase of substrate for the first rate limiting reaction in the purine synthesis *de novo* (20) a reaction

catalysed by phosphoribosylpyrophosphate amidotransferase.

The orally administered adenine could on the one hand consume the possible excess of PRPP by the conversion in adenosine monophosphate and on the other hand strengthen the feedback inhibition via the formed purine nucleotides. In this way we can explain the decrease in the purine synthesis *de novo* we found in patients Re W and Ro W during adenine medication.

The question is still open however whether through the decreased purine synthesis *de novo* the nucleic acid synthesis has improved. The disappearance of the megaloblastic anaemia with adenine therapy at least suggests that at any rate this is the case in the erythrocytes. The question which remains to be answered is whether the improvement of the nucleic acid synthesis also takes place in the cerebral ganglion cells and if this is the case it remains uncertain whether recovery of cerebral damage is possible in our patients.

We continued oral adenine therapy and supplied allopurinol. Up till now we did not see any change in the neurological symptoms in our patients probably because the cerebral damage was irreversible. On the ground of our experience and the findings of Felix & DeMars it seems useful to treat patients with the Lesch Nyhan syndrome from birth with adenine and to add allopurinol to this medication to correct the hyperuricaemia and to prevent the formation of the renal toxic dihydradenine from adenine by xanthine oxidase action (19).

## SUMMARY

A report is given of the clinical and biochemical features of two brothers with the Lesch Nyhan syndrome. A disappearance of the megaloblastic anaemia and a decrease of the purine synthesis *de novo* was found during oral adenine administration. The influence of adenine medication on the disturbed metabolism in this syndrome is discussed. Adenine in combination with allopurinol is suggested as a medication in the Lesch Nyhan syndrome.





Fig 3 Bone marrow of Re W before a during b and 3 months after c termination of adenine administration

family Ru W the megaloblastosis was less pronounced

On the ground of our investigation and those of other authors (1 7 8 11 12 18) we believe that a megaloblastic anaemia is a characteristic symptom of the Lesch-Nyhan syndrome. No indication of a vitamin B<sub>12</sub> deficiency was found in any of the patients with megaloblastic anaemia.

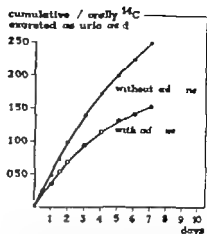


Fig 4 The cumulative excretion of isotope in urinary uric acid after oral gift of glycine-<sup>14</sup>C in patient Re W before and during adenine administration dose adenine 20 mg/kg body weight/day

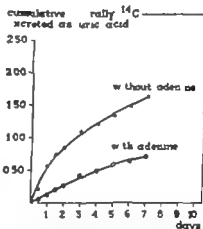


Fig 5 The cumulative excretion of isotope in urinary uric acid after oral gift of glycine-<sup>14</sup>C in patient Ru W before and during adenine administration dose adenine 50 mg/kg body weight/day

Kelley found a decreased folic acid concentration in the serum of his patients (7). Owing to the fact, however, that in a number of patients with a megaloblastic anaemia no folic acid deficiency existed and our patients never

Table 6 The cumulative excretion of isotope in urinary uric acid after oral gift of glycine-<sup>14</sup>C before and during adenine administration

	Re W ( )	Ru W ( )
7 days recovery of isotope in urinary uric acid		
without adenine	2.48	1.63
with adenine	1.53	0.72

## PATTERNS OF MEDICAL AND SOCIAL RESEARCH IN PEDIATRICS

STEPHEN A RICHARDSON

From the Departments of Pediatrics and Community Medicine Albert Einstein  
College of Medicine New York USA

In pediatrics there is heavy emphasis both in training and research on the biology of bodily development, and particularly on pathological conditions in disease and disability. Knowledge about the social conditions that influence children is largely at the common sense level enriched through experience into the art of clinical practice. In the past this was justified by the absence of the social sciences but there is now a body of knowledge concepts and methods which can be brought to bear on social and bio social issues in pediatrics. The purpose of this paper is to illustrate and discuss various ways in which social and medical scientists are working out productive ways of conducting bio social research in pediatrics.

The first illustration deals with a problem identified in pediatric rehabilitation which gave impetus to a variety of studies by social scientists which have clarified the problem and provided some answers. Important pediatric advances in the treatment of physically handicapped children occurred in the decade following World War II such as advances in medical training surgical correction physical and occupational therapy. With these achievements there was also disappointment. Disappointment as it was recognized that after intensive rehab-

ilitation programs persons with physical disabilities were often dropping out of school early were having trouble finding jobs and losing their jobs even though they were technically able to perform the tasks.

A British study of cerebral palsy (7) found

"The most striking feature shared to some extent by all those who require prolonged training is no maturity. It does seem however that these children have been denied experience in many activities which given encouragement they could have enjoyed. Many behave as though they had gone through their childhood on a conveyor belt system which involved no real responsibility or need for awareness of life around them" (pp 93-94).

A study in the United States found (7):

"Cerebral palsyed adults who are potentially employable and capable of social activity are typically unemployed and socially isolated" (p 633).

For the physician success is often measured by the improvement or disappearance of a patient's symptoms as seen during hospital or clinic visits. These two follow up studies focus on the broader issue of the long term consequences of disability for later social functioning.

The following questions are suggested by these studies:

Are there certain social experiences that are essential for young people in order to develop the social skills needed for functioning as a mature adult? Do people with handicaps either miss or only partially share these experiences?

This paper is a revision of an invited paper given at the XII International Congress of Pediatrics, Montreal 1968 under the title "Contributions of Social Science to Pediatrics".

## REFERENCES

- 1 Cattel W & Schmidt J Über familiäre gichtische Diathese in Verbindung mit zerebralen und renalen Symptomen bei einem Kleinkind *Deutsch Med Wochr* 48 2145 1959
- 2 Cartier P & Hamet M Les activités purine phosphoribosyltransferases des globules rouges humaines *Clin Chim Acta* 20 205 1968
- 3 Elkinton J R Huth E J Webster G D & McCance R A The renal excretion of hydrogen ion in renal tubular acidosis I quantitative assessment of the response to ammoniumchloride as an acid load *Amer J Med* 29 554 1960
- 4 Felix J S & DeMara R Purine requirement of cells cultured from humans affected with Lesch Nyhan syndrome (hypoxanthine guaninephosphoribosyltransferase deficiency) *Proc Nat Acad Sci* 62 536 1969
- 5 Kaufman J M Greene M L & Seegmiller J E Urine uric acid to creatinine ratio—a screening test for inherited disorder of purine metabolism *J Pediatr* 73 583 1968
- 6 Kelley W N Seminars on the Lesch Nyhan syndrome Hypoxanthine guanine phosphoribosyltransferase deficiency in the Lesch Nyhan syndrome and gout *Fed Proc* 27 1047 1969
- 7 Kelley W N Greene M L Rosenbloom F M Henderson J F & Seegmiller J E Hypoxanthine guanine phosphoribosyltransferase deficiency in gout *Ann Intern Med* 70 155 1969
- 8 Labrune B Cartier P Hamet M Bonnesfuit F Velin J Rubiette M & Mallet R Encephalopathie familiale avec hyperuricémie *Lancet* Med 76 2337 1968
- 9 Lesch M & Nyhan W L Familial disorder of uric acid metabolism and central nervous system function *Amer J Med* 36 561 1964
- 10 Liddle L Seegmiller J E & Laver L The enzymatic spectrophotometric method for determination of uric acid *J Lab Clin Med* 54 903 1959
- 11 Manzke H Hyperuricémie mit zerebraler Parésie Syndrom eines hereditären purinstoffwechselstoffs *Helv Paediat Acta* 22 258 1967
- 12 Marie J Royer P & Rappaport R Hyperuricémie congénitale avec troubles neurologiques rentux et ainsuins *Arch Franc Pediat* 24 501 1967
- 13 Michener W M Hyperuricemia and cerebral retardation *Amer J Dis Child* 113 195 1967
- 14 Munsat T L Klinenberg J Carrel R & Menkes J Defect in purine metabolism and neurological disease *Bull Los Angeles Neurol Soc* 33 101 1968
- 15 Nyhan W L Oliver W J & Lesch M A familial disorder of uric acid metabolism and central nervous system function II *J Pediatr* 67 25 1964
- 16 Nyhan W L Pesek J Sweetman L Carpenter D G & Carter C H Genesis of a linked disorder of uric acid metabolism and central function *Pediat Res* 1 5 1967
- 17 Nyhan W L Seminars on the Lesch Nyhan syndrome Introduction—clinical features *F Proc* 27 1027 1968
- 18 — Seminars on the Lesch Nyhan syndrome 1968 Summary of clinical features *Fed Proc* 27 103 1968
- 19 Philips F S Thiersch J B & Bendich J Adenine intoxication in relation to its biosynthesis and deposition of 2-8 dihydradenine renal tubulus *J Pharmacol Exp Ther* 104 1952
- 20 Rosenbloom F M Henderson J F Calhoun H C Kelley W N & Seegmiller J E Chemical bases of accelerated purine biosynthesis de novo in human fibroblasts lacking hypoxanthine guanine phosphoribosyltransferase *J Biol Chem* 243 1166 1968
- 21 Royer P *Actualités néphrologiques de l'homme* Ed Flammarion Paris 1968 p 301
- 22 Seegmiller J E Rosenbloom F M & Kelley W N Enzyme defect associated with a linked human neurological disorder and extreme purine synthesis *Science* 155 1682 1967
- 23 Shapiro S L Sheppard G L Dreifuss F J & Newcombe D S X-linked recessive inheritance of a syndrome of mental retardation and hyperuricemia *Proc Soc Exp Biol Med* 1 740 1966
- 24 Zee S P M van der Mondens L A H Schretlen E D A M Een hereditaire purinstofwisselingsstoornis met een cerebrale stoornis en megaloblastaire anemie (Syndroom van Lesch en Nyhan) *Nederl T Geneesk* 11 147 1969
- 25 Zee S P M van der Schretlen E D A M Mondens L A H Megaloblastic anaemia in the Lesch Nyhan syndrome *Lancet* 1 1427 1969

Submitted Aug 29 1969

Accepted Oct 31 1969

(S P M van der Zee)  
University Paediatric Department  
St Radboud Hospital  
Geert Grooteplein 20  
Nijmegen  
The Netherlands

Key words Lesch Nyhan syndrome megaloblastic anaemia adenine

## PATTERNS OF MEDICAL AND SOCIAL RESEARCH IN PEDIATRICS

STEPHEN A RICHARDSON

*From the Departments of Pediatrics and Community Medicine, Albert Einstein College of Medicine, New York, USA*

In pediatrics there is heavy emphasis both in training and research on the biology of bodily development, and particularly on pathological conditions in disease and disability. Knowledge about the social conditions that influence children is largely at the common sense level enriched through experience into the art of clinical practice. In the past this was justified by the absence of the social sciences but there is now a body of knowledge, concepts and methods which can be brought to bear on social and bio-social issues in pediatrics. The purpose of this paper is to illustrate and discuss various ways in which social and medical scientists are working out productive ways of conducting bio-social research in pediatrics.

The first illustration deals with a problem identified in pediatric rehabilitation which gave impetus to a variety of studies by social scientists which have clarified the problem and provided some answers. Important pediatric advances in the treatment of physically handicapped children occurred in the decade following World War II such as advances in medication, bracing, surgical correction, physical and occupational therapy. With these achievements there was also disappointment. Disappointment as it was recognized that after intensive rehab-

ilitation programs persons with physical disabilities were often dropping out of school early, were having trouble finding jobs and losing their jobs even though they were technically able to perform the tasks.

### *A British study of cerebral palsy (6) found*

The most striking feature shared to some extent by all those who require prolonged training is immaturity. It does seem however that these children have been denied experience in many activities which given encouragement they could have enjoyed. Many behave as though they had gone through their childhood on a conveyor belt system which involved no real responsibility or need for awareness of life around them. (pp 93-94)

### *A study in the United States found (7)*

Cerebral pained adults who are potentially employable and capable of social activity are typically unemployed and socially isolated. (p 655)

For the physician success is often measured by the improvement or disappearance of a patient's symptoms as seen during hospital or clinic visits. These two follow up studies focus on the broader issue of the long term consequences of disability for later social functioning.

The following questions are suggested by these studies:

Are there certain social experiences that are essential for young people in order to develop the social skills needed for functioning as a mature adult? Do people with handicaps either miss or only partially share these experiences?

This paper is a revision of an invited paper given at the XII International Congress of Pediatrics, Mexico 1968 under the title: *Contributions of Social Science to Pediatrics*.

Are there special difficulties or barriers which people with physical disabilities encounter in establishing social relationships with other people?

The following studies illustrate ways in which these questions have been explored

Kleck *et al* (8) designed an experiment to determine whether a person who is not handicapped behaves in a different way when he meets a person who is visibly handicapped compared to when he meets a person who is not handicapped. The subjects of the experiment were high school students. Each subject met a handicapped or non handicapped peer whom they had not previously known. To insure that differences in the behavior of the high school subjects were due to the presence or absence of a physical handicap the experiment had to be designed so that apart from the presence or absence of a handicap the appearance, manner and behavior of the handicapped and non handicapped peer were identical. This was accomplished by the experimenters hiring and training a high school boy who acted as their confederate. This confederate was not handicapped but could be made to appear either as a handicapped or non handicapped person. This was done through the use of a wheel chair with a false bottom and by the confederate bending one of his knees and placing his leg below the knee into the false bottom. This successfully created the illusion of a below knee amputation. The confederate was trained to behave in exactly the same way toward every subject whether he appeared as handicapped or non handicapped. Half of the subjects met one at a time with the confederate when he played the role of a normal person. The other half met individually with the confederate when he played the role of a handicapped person. None of the subjects knew of the experimental arrangements. They all thought they were meeting another student subject.

The following ideas were tested in the study. Many people experience some ambivalence of feelings when they first meet a handicapped

person. This will make them fearful of spontaneity in case they reveal their negative feelings. Meeting a handicapped person is also an unfamiliar experience for most people. From this reasoning it was predicted that a non handicapped person in meeting a handicapped person would be more stereotyped, inhibited and overcontrolled and would be more anxious than in encounters with non handicapped persons.

In many countries there is a strong value which dictates kind and considerate treatment of others especially when others are less fortunate than ourselves. From this the investigators postulated that a non handicapped person will distort information in the direction he thinks the handicapped person would like to hear.

The results showed that when the non handicapped subjects were in contact with the handicapped as compared with the non handicapped person they expressed opinions less representative of their beliefs. This suggests that the handicapped person does not receive accurate or spontaneous feedback from others. Absence of accurate feedback makes it difficult for the person who is handicapped to learn appropriate behavior and makes it difficult for him to develop social skills and know what others really think of him.

The non handicapped subjects showed more emotional arousal as measured by the psychogalvanic skin response and were more formal and inhibited in their behavior toward the handicapped person. This reduces the repertoire of behavior to which the person with a handicap is exposed thus limiting the range of his experience in social relationships.

A second experiment used a somewhat different approach (9). There are a wide range of social encounters in which the potential participants have some degree of choice as to whether to initiate or avoid a social encounter. The choice will be influenced by the chooser's attitudes or values toward the person he may meet. Essentially a value is a preference ordering among alternatives. The study of values

was approached by using six drawings of the same child in which everything was held constant except the presence or absence of a visible physical disability and the type of disability. The pictures were laid before the child being tested and he was asked to look at each picture carefully. He was then asked "Which boy do you like best?" When the child indicated his preference this picture was then removed. He was asked again "Now which boy do you like best?" and the procedure was repeated until only one picture remained. In this way a rank ordering of each child's preferences was obtained.

The initial study was of 640 children aged 9-11 in the United States. The main findings were that the children preferred the non handicapped child to the five children with various handicaps. The same finding has since been obtained for children in Germany, Greece, Israel and Mexico. The children exhibited a remarkable degree of agreement in their preference order of the different handicaps. The order from most to least liked was: 1) A child without a visible handicap, 2) A child with crutches and a brace on the left leg, 3) A child in a wheelchair, 4) A child with a left forearm amputation, 5) A child with a slight facial disfigurement, 6) An obese child. The children with physical handicaps that were tested displayed the same values as children who were not handicapped. A rather sad result.

These findings suggest that children's values towards disability will make them less inclined to initiate social relations with a handicapped than a non handicapped child. Another study of friendship patterns show handicapped children are less likely to be chosen as friends than non handicapped children (2).

These studies provide evidence suggesting the following answers to the general questions posed earlier.

Children with handicaps do mix or only partially share in certain social experiences that are essential to develop the social skills needed for functioning as an adult.

There are special difficulties or barriers

which children with physical disabilities encounter in establishing social relationships with others.

These and other studies (10) suggest that it is as important for a handicapped child to receive special training in social skills as it is for him to receive the more traditional rehabilitation services which have focused on bodily pathology. There have of course been recreational programs planned for handicapped children but these have been hampered by lack of specific knowledge of the kinds of experiences the children have had, the kinds of skills they most need to learn, the techniques for dealing with the social obstacles they will encounter because of their disability and how these skills are best taught. While knowledge from social science studies is still fragmentary and partial it is growing quickly. There are social rehabilitation techniques that could now be usefully employed e.g. some specific techniques that are used by handicapped persons in breaking through the social barriers created by their disabilities (3) and these could be taught using role playing and other training techniques.

A second illustration of two social research is a finding of a medical epidemiologist that raises a challenging question for social scientists. Gruenberg (5) examined the prevalence of mental subnormality at different age levels. Despite the differences found in various studies there was agreement on one striking finding.

After the age of fourteen the prevalence is rarely half as high as at the age of fourteen. For this to occur a large group of people regarded as retarded at fourteen must improve in their functioning to the point where people no longer regard them as retarded and also succeed in escaping their history of earlier unsatisfactory performance.

Either these individuals are continuing to be extremely handicapped in later life and are unknown because the services they need are unavailable to them, or they have stopped being retarded in any real sense at all and do not need any special protection, help or services in which case one had better change one's concept of what real mental retardation really is (pp 273-274).

A study by Edgerton (4) throws some light on the question. He followed up 48 adults who

had been inmates of a large state institution for the mentally retarded, and had been discharged having been judged able to conduct their lives in the community without supervision. Their mean IQ was 66 with a range from 48-84. Using intensive interviews Edgerton obtained a detailed picture of their day to day lives and of some of the difficulties they encountered.

The critical problem the ex patients faced after release from the hospital was the continued attempt to hide their incompetence from others and to try to pass as normal people. Ex patients enter the outside world without any of the possessions which normal people accumulate. To the ex patients these possessions are essential symbols of being normal in the outside world. They try to remedy this by requiring souvenirs: photo albums and oddments from junk shops, trash cans and friends. In addition to fabricating a past to conceal their real history they acquire new souvenirs of their lives after release and display them proudly. Although they cannot read adequately they buy books and magazines and display them prominently in their homes. Because they receive little or no mail they try and give the appearance of doing so by keeping and displaying what they do receive and sometimes collecting other people's discarded letters.

In city life there are a variety of situations where everyone is called upon to read—using telephones, bus destinations, shopping etc. The ex patient develops excuses for hiding his incompetence in reading to others: e.g. he says to a normal person that he's forgotten his glasses and can't see the words in question (p 164). The obliging normal then reads for him.

The use of numbers presents a difficult challenge. For example telling time. One device used is to wear a watch that is not running. They can then ruefully look at their watches and say 'My watch stopped. But to ask

What time is it?' can lead to replies they can not understand such as 'It's twenty to nine' or 'It's eight forty'. To avoid confusion a

number of techniques were used, such as asking in numbers they could understand, such as 'Is it nine o'clock yet?'

One essential component for coping with the problems of living in the community is having a benefactor—a normal person who helps them cope with everyday problems, helps them hide their areas of incompetence and avoid getting into trouble. Various people such as landladies, neighbors, employers and spouses acted as benefactors. The majority knew the ex-patient's past, and helped with problems of biography management and concealment of the past. They helped conceal deficits in everyday competence such as appearance, dress or speech and inability to read, write or deal with numbers.

Another critical problem for the ex patients was the building up and maintenance of their self esteem. However well the ex patients develop such devices to pass as normal people they cannot fail to realize that their competence in many aspects of everyday life is clearly less than that of the normals with whom they must associate. Such a realization is potentially devastating to their self esteem and if the integrity of the self is to be maintained imputations of stupidity must be denied. The process of denial is continuous (p 169). Many of them appear to believe that they are relatively less competent than normal people because they have suffered the depriving experience of having been confined—wrongly of course—in Pacific State Hospital (p 169).

In the ex patients' efforts to establish their worth as normal human beings they are greatly in need of the affection and respect of normal persons (p 201). Benefactors often help to supply this want. In order to convey affection without seeming to be patronizing the normal benefactor must be highly sensitive to the ex patient's need for self respect (p 201).

Edgerton also brings out the terrible stigma which the term mentally retarded imposes on those who are so designated by the society. This stigma has been imposed and reinforced

from childhood on. Prior to being placed in the State Hospital Edgerton's subjects had been repeatedly told directly or indirectly that their intelligence was deficient. Parents, peers, teachers, neighbors, and even strangers presented a consistent refrain of rejection and humiliation (p. 146). The patient was then "accused and found guilty of being so stupid that he was considered incompetent to manage his own life" (p. 145) and was committed to a mental hospital.

It is clear there are services that these ex-patients need. The dilemma is how can these services be provided on an organized basis without stripping away the disguise these mentally retarded adults so struggle to put on and maintain in order to hide their incompetence, pass as normal and maintain some self-esteem. In the future this dilemma can only be resolved if we reduce the frequency with which mentally subnormal children and youth experience failure, rejection and humiliation. This requires a careful rethinking of present services for children and youth. For example, the peak of prevalence for mental subnormality found in the last years of school strongly suggests that the intellectual demands made on young people are greater than those that many of them will ever again have to face. We need to consider the cost of frequent failure in day to day school life for large numbers of children and what this does to their self-esteem and future development.

The two illustrations given are concerned with issues where further understanding is greatly facilitated by closer communication and working together of pediatricians, social scientists and educators. One of the serious obstacles to cooperation between the social and medical sciences has been their isolation one from the other with little common training, communication or mutual education. The lack of close working relations between the social and medical sciences is most serious in dealing with the numerous problems which concern pediatrics which neither the biological nor social scientists can tackle fully alone but de-

mand both sets of research skills. For example, childhood accidents which are one of the major causes of morbidity and mortality, the causes and the consequences of malnutrition, mental subnormality, poverty, the battered child syndrome, and the child who has educational handicaps.

A community study of mental subnormality will be used as an illustration of bio-social co-operative research (1).

A goal of the study was to explore the relative contribution of social, biological and genetic factors to the causes of mental subnormality and its subtypes. One step toward this goal was to examine the kinds of families where there are mentally subnormal children. The children studied were the total population of mentally subnormal children aged 8-10 resident in Aberdeen, Scotland. From the total population of 104 children there were 6 where the causes had been clearly identified—5 with a known genetic origin—Down's syndrome—and one child where the subnormality was clearly the consequence of severe meningococcal cephalitis in the third year of life.

The families of the remaining 98 children where the causes of MS were unclear were classified by the occupation of the father or head of the household. The occupations were then grouped into the broad social class classifications used by the Registrar General. The children were grouped into subtypes based on IQ score and the presence or absence of clinical evidence of CNS damage. For the MS children having IQs of less than 50 and clear evidence of CNS damage there are sound grounds in the clinical findings of brain damage for believing that the mental subnormality derived primarily from CNS damage. These children are randomly distributed across social classes (Table 1). This provides no reasonable basis for inferring that social environment or familial constitutional factors have made a significant contribution to the production of the handicap.

The least severely subnormal children are those with IQ of 60 or greater and without



had been inmates of a large state institution for the mentally retarded and had been discharged having been judged able to conduct their lives in the community without supervision. Their mean IQ was 66 with a range from 48-84. Using intensive interviews Edgerton obtained a detailed picture of their day to day lives and of some of the difficulties they encountered.

The critical problem the ex-patients faced after release from the hospital was the continued attempt to hide their incompetence from others and to try to pass as "normal people". Ex-patients enter the outside world without any of the possessions which normal people accumulate. To the ex-patients these possessions are essential symbols of being normal in the outside world. They try to remedy this by acquiring souvenirs, photo albums and oddments from junk shops, trash cans and friends. In addition to fabricating a past to conceal their real history they acquire new souvenirs of their lives after release and display them proudly. Although they cannot read adequately they buy books and magazines and display them prominently in their homes. Because they receive little or no mail they try and give the appearance of doing so by keeping and displaying what they do receive and sometimes collecting other people's discarded letters.

In city life there are a variety of situations where everyone is called upon to read—using telephones, bus destinations, shopping etc. The ex-patient develops excuses for hiding his incompetence in reading to others: e.g. he says to a normal person that he's forgotten his glasses and can't see the words in question (p. 164). The obliging normal then reads for him.

The use of numbers presents a difficult challenge. For example telling time. One device used is to wear a watch that is not running. They can then ruefully look at their watches and say "My watch stopped". But to ask "What time is it?" can lead to replies they can not understand such as "It's twenty to nine" or "It's eight forty". To avoid confusion a

number of techniques were used, such as asking in numbers they could understand such as "Is it nine o'clock yet?"

One essential component for coping with the problems of living in the community is having a "benefactor"—a normal person who helps them cope with everyday problems, helps them hide their areas of incompetence and avoid getting into trouble. Various people such as landlords, neighbors, employers and spouses acted as benefactors. The majority knew the ex-patient's past, and helped with problems of biography management and concealment of the past. They helped conceal deficits in everyday competence such as appearance, dress or speech, and inability to read, write or deal with numbers.

Another critical problem for the ex-patients was the building up and maintenance of their self-esteem. However well the ex-patients develop such devices to pass as normal people,

they cannot fail to realize that their competence in many aspects of everyday life is clearly less than that of the normals with whom they must associate. Such a realization is potentially devastating to their self-esteem and if the integrity of the self is to be maintained, imputations of stupidity must be denied. The process of denial is continuous (p. 169). Many of them appear to believe that they are relatively less competent than normal people because they have suffered the depriving experience of having been confined—wrongly of course—in Pacific State Hospital (p. 169).

In the ex-patients' efforts to establish their worth as normal human beings they are greatly in need of the affection and respect of normal persons (p. 201). Benefactors often help to supply this want. In order to convey affection without seeming to be patronizing the normal benefactor must be highly sensitive to the ex-patient's need for self-respect (p. 201).

Edgerton also brings out the terrible stigma which the term "mentally retarded" imposes on those who are so designated by the society. This stigma has been imposed and reinforced

normality are exposed to a combination of maternal obstetrical and social sub-optimal conditions in their early development. Etiology is more likely to derive from some combination of social-environmental and familial constitutional factors existing in conjunction with ill defined reproductive inadequacy rather than from any definite obstetrical risk condition for CNS insult. Apart from the initial identification of 5 cases with known genetic causes there was no way in the design of the study to fully distinguish between genetic and social environmental etiology.

These examples of research illustrate ways social and medical scientists can cooperate in pediatrics. One approach has been the selection by social scientists of questions raised by physicians which can be taken up and effectively pursued using the special skills of the social scientists: a body of related knowledge, a pertinent conceptual framework and special methods for studying social phenomena. There are some problems in pediatrics where social scientists can work effectively providing pediatricians contribute some minimum cooperation and goodwill. But there are many research problems in pediatrics where more intensive and continuous cooperation is necessary between social and medical scientists and where they must become full working partners. In these cases each must fully contribute his special knowledge and skills to the formulation, course and results of the research so that the product will be different from what either could achieve alone. This form of cooperation in research often takes years to evolve between the research partners requiring a mutual education to find out how best to use their respective research and writing skills.

The pediatrician has settings and opportunities in the hospital, his office and on house calls to gain access to behavior of the neonate child and adolescent and to some extent to his parents' families. This range of behavior in health and more particularly during illness and disability provides valuable opportunities for observations and insight from which the social

scientist can gain. Once a question has been raised however the pursuit of the question may take the social scientist into social settings rarely seen by the physician—into the classroom, the playground, the summer camp. Just as the pediatrician can enrich the experience and research opportunities of the social scientist so the social scientists can also help the pediatrician. The social scientist through differences in viewpoint and in the settings in which he works can raise research issues that may not have occurred to the pediatrician, questions about medical practices which have become habitual and which may no longer serve an appropriate function.

Starting with the great bacteriological discoveries of the nineteenth century medicine sought specific causes for specific disorders. This single factor approach to research then came to be seen as inadequate. There is a growing recognition that more complex multifactorial conceptual models are necessary and that many diseases and disabilities are the outcome of a complex process over time involving biological, genetic and social environmental components.

In summary there are two broad categories of research in which the social and medical scientist must work together. One is in furthering understanding of the two social factors which contribute to the causes and consequences of disease and disability. The other is in the planning, conduct and evaluation of health services.

The long and almost complete separation of the social and medical sciences is being complemented with increasing speed by various forms of cooperation and collaboration. These forms of bio social research are essential to obtain the knowledge necessary to take seriously the goal of the World Health Organization as reflected in their definition of health: a state of complete physical, mental and social well being, as well as being the absence of disease or infirmity.

Table 1 *Social class distribution of mentally subnormal children with  $IQ < 50$  and clinical evidence of central nervous system damage*

Social class	Actual no	Expected no	Actual no / Expected no *
I-IIIa (2405)	7	6.80	1.03
IIIb (1904)	4	5.41	0.74
IIIc (1648)	5	4.70	1.28
IV (1087)	3	3.10	0.97
V (1044)	3	2.90	1.03
Total (8058)	23	23	1.00

Total at risk in social class  $\times$  Total MS  $< 50$  with CNS damage / Total at risk in total population

\* A figure greater than 1.0 indicates a higher concentration and a figure less than 1.0 a lower concentration of cases than expected

evidence of CNS damage. It is this subtype where the literature abounds in diverse opinions about etiology, because at present there is no clear evidence for attributing cause to any one of the general etiological factors. There are no children with this least severe subtype of subnormality among the upper social class families—where the father is in a non manual journeyman or artisan occupation (Table 2). By contrast these children are heavily overrepresented in the semi skilled manual and especially in the unskilled manual occupations, the lowest social classes.

Social class is a very general indicator for characterizing the life styles of families. To learn more about the lower class families where the least severe subtype of subnormality is so heavily concentrated a number of additional family characteristics were examined.

Table 2 *Social class distribution of children with  $IQ \geq 60$  without clinical evidence of central nervous system damage*

Social class	Actual no	Expected no	Actual no / Expected no
I-IIIa (2405)	0	11.3	—
IIIb (1904)	0	8.9	—
IIIc (1648)	8	7.7	1.0
IV (1087)	12	5.1	2.4
V (1044)	18	4.9	3.7
Total (8058)	38		

The families in the lowest social classes with children who are minimally subnormal are overrepresented when the following features are present: 1) Five or more children; 2) Residence in a poor housing area; 3) Living under more crowded conditions as measured by person room ratio; 4) Where the mother held a semi- or unskilled occupation prior to marriage; 5) A higher frequency of siblings that are mentally subnormal compared to families of the same social classes where there is not an 8-10 year old MS child.

These family characteristics are not, of course, factors which cause minimal subnormality. Rather they characterize the kinds of families where there is a higher risk of minimal subnormality. Knowing these characteristics provides a basis for further more intensive studies on a carefully defined population. The results also provide a basis for speculative inference suggesting guilt by association. On a inferential grounds there is evidence to support the conclusion that social environmental factors are contributing to this least severe clinical subtype of MS.

For the same group of minimally subnormal children the obstetrical and perinatal histories of the mother and infant were compared with a comparable population of children who were not mentally subnormal.

In complications of pregnancy the mothers of the minimally subnormal children did not experience severe or moderate pre-eclampsia more often than mothers of the comparison population but did have a higher frequency of antepartum hemorrhage and threatened abortion when both types of bleeding are combined. There were no significant differences in either duration of labor or in types of presentation or delivery. There was a higher proportion of minimally subnormal children with birth weights under 6 lbs and gestational ages of 37 weeks or under. A higher proportion of the mothers of these children were 5 ft in height or under.

These findings suggest that these children in the mildest clinical subtype of mental sub-

normality are exposed to a combination of maternal, obstetrical and social sub-optimal conditions in their early development. Etiology is more likely to derive from some combination of social-environmental and familial constitutional factors existing in conjunction with ill defined reproductive inadequacy rather than from any definite obstetrical risk condition for CNS insult. Apart from the mutual identification of 5 cases with known genetic causes there was no way in the design of the study to fully distinguish between genetic and social environmental etiology.

These examples of research illustrate ways social and medical scientists can cooperate in pediatrics. One approach has been the selection by social scientists of questions raised by physicians which can be taken up and effectively pursued using the special skills of the social scientists: a body of related knowledge, a pertinent conceptual framework and special methods for studying social phenomena. There are some problems in pediatrics where social scientists can work effectively providing pediatricians contribute some minimum cooperation and goodwill. But there are many research problems in pediatrics where more intensive and continuous cooperation is necessary between social and medical scientists and where they must become full working partners. In these cases each must fully contribute his special knowledge and skills to the formulation, course and results of the research so that the product will be different from what either could achieve alone. This form of cooperation in research often takes years to evolve between the research partners, requiring a mutual education to find out how best to use their respective research and writing skills.

The pediatrician has settings and opportunities in the hospital, his office and on house calls to gain access to behavior of the neonate, child and adolescent and to some extent to his parents' families. This range of behavior in health and more particularly during illness and disability provides valuable opportunities for observations and insights from which the social

scientist can gain. Once a question has been raised, however, the pursuit of the question may take the social scientist into social settings rarely seen by the physician—into the classroom, the playground, the summer camp. Just as the pediatrician can enrich the experience and research opportunities of the social scientist, so the social scientists can also help the pediatrician. The social scientist through differences in viewpoint and in the settings in which he works can raise research issues that may not have occurred to the pediatrician, questions about medical practices which have become habitual and which may no longer serve an appropriate function.

Starting with the great bacteriological discoveries of the nineteenth century medicine sought specific causes for specific disorders. This single factor approach to research then came to be seen as inadequate. There is a growing recognition that more complex multifactorial conceptual models are necessary and that many diseases and disabilities are the outcome of a complex process over time involving biological, genetic, and social environmental components.

In summary there are two broad categories of research in which the social and medical scientists must work together. One is in furthering understanding of the bio-social factors which contribute to the causes and consequences of disease and disability. The other is in the planning, conduct and evaluation of health service.

The long and almost complete separation of the social and medical sciences is being complemented with increasing speed by various forms of cooperation and collaboration. These forms of bio-social research are essential to obtain the knowledge necessary to take seriously the goal of the World Health Organization as reflected in their definition of health "a state of complete physical, mental and social well-being, as well as being 'the absence of disease or infirmity'."

## REFERENCES

- 1 Birch H G Richardson S A Baird D Horobin G & Illsley R *Mental subnormality—a community study of children* Williams and Wilkins Co Baltimore In Press
- 2 Centers L & Centers R Peer group attitudes toward the amputate child *J Soc Psychol* 61 127 1964
- 3 Davis F Deviance disavowal and normalization *Social Problems* 9 120 1961
- 4 Edgerton II *The cloak of competence* Univ of California Press 1967
- 5 Gruenberg M Epidemiology in H A Stevens & R Heber (eds) *Mental retardation* Univ of Chicago 1964
- 6 Ingram J E *Living with cerebral palsy* Studies in Medicine No 14 The Spastics Society in association with Wm. Heinemann (Medical) Books Ltd London 1964
- 7 Happer Z S & Birch H G The relation of childhood characteristics to outcome in young adults with cerebral palsy *Develop Med Child Neurol* 18 645 1966
- 8 Kleck R Ono H & Hastorf A H The effects of physical deviance upon face to face interaction *Human Relations* 19 425 1966
- 9 Richardson S A Goodman N Hastorf A H & Dornbush S M Cultural uniformity in reaction to physical disabilities *Amer Sociol Rev* 26 241 1961
- 10 Richardson S A The effects of physical disability on the socialization of a child in D A Goslin (ed) *Handbook of socialization theory and research* Rand McNally and Co Chicago 1969

Submitted Nov 21 1969

Accepted Nov 27 1969

Dept of Pediatrics  
 Albert Einstein College of Medicine  
 1300 Morris Park Avenue  
 Bronx N Y 10461  
 USA

Key words Social research paediatrics

## INTESTINAL MALABSORPTION A CLINICAL STUDY OF 22 CHILDREN OVER 2 YEARS OF AGE

J. A. VISAKORPI, P. KUITUNEN and P. PELLONEN

From the Children's Hospital, University of Helsinki, Helsinki, Finland

A classic clinical picture and steatorrhea with gluten intolerance are the criteria usually applied in the diagnosis of coeliac disease. Hence it is difficult to detect the existence of atypical symptomatology in the disease. Nevertheless more or less atypical forms have been described by many authors, especially in older children (2, 3, 8). When the finding of villous atrophy of the intestinal mucosa is taken as the diagnostic criterion of coeliac disease, it is possible to evaluate the clinical manifestations in coeliac disease and even detect other conditions associated with intestinal mucosal villous atrophy.

This report presents the clinical symptomatology and findings in respect of 22 children over 2 years of age in whom significant villous atrophy was found. The authors believe that these patients are all suffering from coeliac disease although this name has not been applied because the diagnosis has not been properly verified in each case. We have previously presented a series of infants with similar findings (11).

### PATIENTS

The patients in the series were children over 2 years of age with partial or subtotal villous atrophy of the duodenoduodenal mucosa. The series comprised all such cases observed during a 7 year period (1962-1969) at the Children's Hospital, University of Helsinki (admission rate approximately 7000/year). Fig. 1 indicates the age of the patients on admission. During the same 7 year period at which these patients were found villous atrophy was also verified in 110 infants under 2 years of age.

Added by grant from the S. and J. Foundation

Biopsy was performed in cases in which the malabsorption syndrome was suspected on the basis of the clinical picture and the results of laboratory tests.

Of the 22 patients 15 were female, such a preponderance of females has been noted in some other series (1) but not in all (3).

The incidence of coeliac disease and of diabetes mellitus were higher among the relatives of these 22 patients than in the general population. Of the 73 siblings two suffered from coeliac disease and two from diabetes mellitus. In addition two had a severe allergic disorder. All the 44 parents were healthy in these respects but in seven families there was diabetes mellitus and in one family coeliac disease in other relatives. The birth weights of the patients were normal and weaning had usually taken place between 2 weeks and 4 months. Four patients however were breast fed for more than 6 months and 2 patients even up to the age of 1 year.

### METHODS

The laboratory examinations reported were performed by the routine methods we have described earlier (10). The biopsies were made by multipurpose suction biopsy tube and with a pediatric Crosby capsule from the distal duodenum or the proximal jejunum. The biopsies were evaluated by conventional light microscopy and the villi were measured (5). The following classification was adopted: normal = median villous length exceeding 300  $\mu$ , slight changes = length 250-300  $\mu$ , partial villous atrophy (PVA) = length 150-250  $\mu$ , subtotal villous atrophy (SVA) = length under 150  $\mu$ . In addition observation was made on the epithelial cell layer and cell infiltration in the lamina propria.

### SYMPTOMS AND SIGNS

In 15 patients the first gastrointestinal symptoms had occurred before the age of 2 years (Fig. 1). These initial symptoms were prolonged or severe watery diarrhoea, sometimes

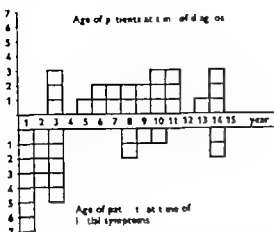


Fig. 1 Ages of patients at time of diagnosis and at time of onset of initial symptoms. Diagnoses made before 2 years of age are not included in this series.

pale and bulky stools and failure to thrive. Coeliac disease was suspected in some cases, although no definitive diagnosis was made. However, these initial symptoms subsided more or less completely in 14 cases, and in consequence the diagnosis was postponed. In 5 cases the apparent cause of this remission was short-term treatment with a gluten-free diet, whereas in the others it occurred spontaneously. Table 1 indicates the presenting symptoms. In 7 cases suspicion was aroused at a routine check by the school physician or on some similar occasion. Table 2 presents all the symptoms observed by the patients or their parents.

Table 1 Main symptoms which drew the attention of the parents (A) or the physician (B) to the illness

	No. of cases
<b>A First symptoms observed by the parents</b>	
Abnormal stools and retarded growth	3
Abdominal pain	2
Anorexia	2
Convulsive seizures	2
Diarrhoea and tetany	1
Diarrhoea and haemorrhage	1
Diarrhoea and abdominal pain	1
Abdominal pain and tetany	1
Vomiting	1
Lassitude	1
<b>B First symptoms found by a physician at the child welfare clinic or on school examination</b>	
Retarded growth	4
Pallor, anaemia	3

Table 2 Symptoms observed by the patients themselves or by their parents

Symptoms	No. of patients
Psychic tiredness and/or irritability	13
Anorexia	9
Abdominal pain	8
Pale, bulky and offensive stools	8
Retarded growth	7
Vomiting	6
Failure to gain weight	5
Watery diarrhoea, recurrent	5
Pallor	4
Constipation	4
Abdominal distention	3
Headache	3
Dislike of wheat	3
Tetanic cramps	2
Oedema	2

and in a single case haemorrhage, rectal prolapse, nausea, hypertrophic scars, conjunctival irritation, telangiectases, sensitivity to infections and loss of hair.

These results demonstrate the relative rarity of the typical coeliac picture, the great variability of the symptoms, and the existence of cases with very few and subjectively minimal symptoms.

Three of these 22 patients suffered concomitantly from diabetes mellitus and in addition one of them from autoimmune thyroiditis. The chronological relationships of these diseases are indicated in Table 3. In addition, one of the patients suffered from a convulsive disorder with intracranial calcification suggesting the Sturge-Weber syndrome; one suffered from Bechterew's prurigo, and in one case a  $\beta_2$ -globulinaemia was found at the same time as the intestinal disease.

As a rule, the clinical findings on admission were rather sparse. Retardation of growth was noted in every case (Fig. 2). However, 10 patients were within two standard deviations of the mean in both height and weight, although their growth curves exhibited a chance of pattern. The heights were more often subnormal than the weights. The abdomen was somewhat enlarged in 13 cases but only in 6 cases markedly so. Haematomas were found in 1 patient and emphysematous chest in 2. Diar-

Table 3 Chronological order of the onset of main symptoms and diseases in three patients suffering concomitantly from intestinal disease and endocrinopathies

Patients	Age at onset of first intestinal symptoms	Age at diagnosis of		
		Malabsorption	Diabetes mellitus	Thyroiditis
M H	2 y 6 mo	10 y 2 mo	1 y 9 mo	10 y
H T	under 1 y	9 y 3 mo	8 y 6 mo	
H N	1 y 2 mo	10 y 8 mo	1 y 4 mo	

rhoea was observed in 4 cases on admission and constipation in 3. In all the other 15 cases the frequency of stools was normal, the quality of stools however was not investigated systematically. In one case convulsions were attributable to hypoglycaemia and in another to concurrent brain disease (Sturge-Weber).

### LABORATORY EXAMINATIONS

The results of laboratory X-ray and histopathological examinations are presented in Table 4 and Fig. 3. As was expected the faecal fat excretion was almost constantly increased (Fig. 3). The D-xylose test was abnormal however in about 50% of cases only. In particular all the diabetes had a normal excretion of D-xylose.

The results of laboratory tests demonstrated that false negative results were derived from every test employed for the indication of malabsorption viz faecal fat, the D-xylose excretion test, FIGLU test, precursors to cow's milk and gluten and barium meal. Nevertheless

every patient had an abnormal result in at least two of these tests.

One group of examinations was concerned with investigation of the nutritional defects of the patients. Anaemia, in particular iron-deficiency anaemia, was the commonest nutritional defect after retarded growth. In some cases it was very severe (Fig. 3). In four cases megaloblastic features were combined with the iron deficiency. In every case this was due to

Table 4 Laboratory X-ray and histopathological findings

Since not all the patients were successfully examined with all the tests, the numbers of those examined are indicated. All tests except biopsy were made before treatment was started.

Faecal fat excretion more than 5 g/day	18/22
Fat absorption less than 9%	17/21
Abnormal fat excretion and/or absorption	19/22
D-xylose excretion less than 20	11/21
Urinary FIGLU positive	17/20
Haemoglobin less than 10 g/100 ml	12/22
Haemoglobin less than 11 g/100 ml	19/22
Serum iron less than 40 µg/100 ml	16/1
Megaloblastic features in bone marrow or peripheral blood	4/22
Prothrombin less than 80	7/19
Serum calcium less than 4 mEq/l	2/17
Serum alkaline phosphatase activity more than 10 B.L. units	2/17
Genitital aminoacids	2/20
Normal sweat chloride	20/20
Serum total protein less than 6.5 g/100 ml	6/16
Decrease of serum IgA during treatment	3/11
Absent serum IgA	1/22
Precursors to cow's milk	15/18
Precursors to gluten	9/18
Barium meal malabsorption pattern	15/16
Double X-ray retarded bone age	11/11
Duodenogastric biopsy	
Subtotal villous atrophy	17/22
Partial villous atrophy	5/22

Three of these were not successfully biopsied until 1-2 years after the start of therapy.

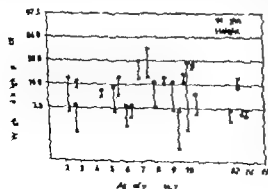


Fig. 3 Weight and height of patients at the time of admission.



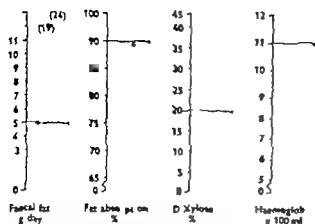


Fig 3 Results of determination of faecal fat excretion estimation of fat absorption D-xylose tests and determinations of blood haemoglobin

these cases) The other nutritional defects clinically manifest were hypocalcaemic tetany (in two cases) rickets (in two cases not the same as the preceding) hypoprothrombinaemic haemorrhage (in one) hypoglycaemic convulsions (in one) and hypoproteinaemic oedema (in one)

As a rule, intestinal biopsy showed a typical flat mucosa with round cell infiltration and an abnormal epithelial cell layer Two of the patients with partial villous atrophy had not undergone any preceding therapy

## RESULTS OF TREATMENT

folic acid deficiency (according to the measurement of serum folic acid and vitamin B<sub>12</sub> in

For study of the possible role of iron deficiency in mucosal damage (7) 2 patients (patients

Table 5 Results of treatment with gluten free diet in 20 patients

In 3 cases (A M P M L & L O) two separate periods of treatment were concerned

Duration of diet	Responses to diet <sup>a</sup>			
	Growth	Absorption	Mucosa	IgA
<i>Strictly gluten free diet</i>				
I I 8 mo	+	NS	NS	-
S L P 1 y	+	NS	NS	+
A M P diet 2 1 y	+	NS	NS	-
R R 1 y 1 mo	+	NS	NS	-
K S 1 y 10 mo	+	NS	NS	-
M U 2 y 5 mo	+	+	+	-
H K 2 y 7 mo	+	+	-	Absent
S k 3 y	+	+	-	NS
H T 3 y 9 mo	-	+	-	+
T M 4 y 1 mo	-	+	-	NS
(J P 3 y 5 mo) <sup>b</sup>	+	±	±	NS
J P 5 y 10 mo	+	+	-	NS
<i>Poorly controlled gluten free diet</i>				
J F 2 y 1 mo	-	±	-	-
M L diet 2 2 y 7 mo	+	±	-	-
L O diet 2 3 y 4 mo	+	-	-	-
T k 3 y 7 mo	-	+	-	-
S E 3 y 10 mo	+	+	-	-
<i>Gluten free + unrestricted diet</i>				
M L diet 1 1 y 0 mo 2 y 4 mo	-	NS	NS	NS
A H 2 y 0 mo + 0 y 4 mo	+	-	-	-
F k 2 y 0 mo + 0 y 11 mo	+	-	-	-
H N 5 y 5 mo + 0 y 1 mo	-	+	-	NS
A M P diet 1 3 y 2 mo + 2 y 1 mo	+	-	-	NS
L O diet 1 0 y 6 mo + 2 y 3 mo	+	-	-	NS
M H 3 y 0 mo + 0 y 7 mo	+	-	-	-

<sup>a</sup> Explanations of symbols in table + = full normalisation + = improvement - = no change NS = not studied

<sup>b</sup> Initial biopsy failed

Hypothyroid patient treated with thyroxine

<sup>c</sup> The patient J P was investigated twice after periods of different lengths

(U and S E in Table 5) with late onset of the disease no typical coeliac symptoms and overt iron-deficiency anaemia were initially treated with iron alone for 3 years and 6 months respectively and subsequently re-examined. No improvement in absorption function or mucosal damage was observed.

Treatment with gluten free diet (rice and corn being the only cereals allowed) was then started in every case. Table 5 presents the results obtained. Follow up examinations were made in 20 cases; however in 1 patient with hypothyroidism induced by thyroiditis (M II) no conclusion could be drawn in view of the impracticability of distinguishing the effects of the gluten free diet and the thyroidism given simultaneously. In others (19 cases) a good clinical response especially in regard to growth was found in 17 cases, improvement in absorptive function in 11 of the 16 cases tested and improvement in the intestinal mucosa in 3 cases out of 13. Some response in respect of either growth, absorptive function or mucosa was found in all 19 cases.

The patients on a strictly gluten free diet invariably displayed a clinical response: normalisation of the absorptive function and in some even normalisation of the mucosa although not until treatment had been continued for 2-5 years. Serum IgA showed a decrease in many patients. Precipitins to gluten disappeared in all but 1 case but precipitins to cow's milk persisted in 4 of the 10 patients who were initially found to have precipitins.

The patients on a poorly controlled gluten free diet although with restricted gluten were all also in good clinical condition, growing well and even exhibiting improvement in the absorptive function. The mucosal damage did not heal in this group and the serum IgA level remained unchanged. Precipitins to gluten were also found during treatment in 1 patient.

The third group includes cases in which the patients were initially on a gluten free and then on a more or less normal diet. All except 1 patient showed initially a response in growth but premature discontinuation of dietary treat-

Table 6 Development of serum IgA concentration and precipitins to cow's milk and to gluten when a relapse was developing in a patient (A V P)

The patient was initially treated with a poorly controlled gluten free diet for 3 years and a month with a rather good clinical response. Then 2 years 1 month before the start of a new diet period the patient was started on an unrestricted diet. A change in the growth pattern and anorexia were the only clinical symptoms. In the table the time of beginning a new gluten free diet is denoted by 0.

	Time			
	-2y	-1y	0	+5 mo
Serum IgA mg/100 ml	22	90	126	16
Precipitins to cow's milk	-	+	-	-
Precipitins to gluten	-	-	+	-

ment arrested the progress towards recovery. The final results were dependent on the length of the period of gluten-containing diet. All 3 patients kept on a gluten-containing diet for more than 2 years suffered clinical relapse. The clinical relapses were manifested by a change in the growth pattern. In addition 1 patient had convulsions of unknown etiology (hypoglycaemia?). The serum IgA was high in all three during the relapse and precipitins were found. Two of the others displayed an increase in serum IgA and an increase in precipitins some months after the start of the unrestricted diet. In 1 case these immunological variants were followed when clinical relapse was developing (Table 6).

#### COMPARISON OF MANIFESTATIONS AND RESPONSES TO THERAPY IN PATIENTS OF DIFFERENT TYPES

It was found that the patients with diabetes mellitus and a  $\gamma$ A globulinaemia all had some typical coeliac symptoms. Absorption tests revealed clear malabsorption except that the diabetics had a normal xylose test. The diabetics responded rather slowly to the diet and normalisation of the mucosa was not observable in these patients but the diet was strict in only 1 patient out of 3. However the patient with

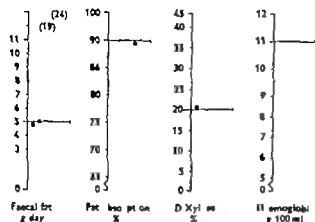


Fig 3 Results of determination of faecal fat excretion, estimation of fat absorption, D-xylose tests and determinations of blood haemoglobin

these cases) The other nutritional defects clinically manifest were hypocalcaemic tetany (in two cases) rickets (in two cases, not the same as the preceding) hypoprothrombinaemic haemorrhage (in one) hypoglycaemic convulsions (in one) and hypoproteinaemic oedema (in one)

As a rule intestinal biopsy showed a typical flat mucosa, with round cell infiltration and an abnormal epithelial cell layer Two of the patients with partial villous atrophy had not undergone any preceding therapy

## RESULTS OF TREATMENT

folic acid deficiency (according to the measurement of serum folic acid and vitamin B<sub>12</sub> in

For study of the possible role of iron deficiency in mucosal damage (7) 2 patients (patients

Table 5 Results of treatment with gluten free diet in 20 patients

In 3 cases (A M P M L & L O) two separate periods of treatment were concerned

Duration of diet	Responses to diet <sup>a</sup>			
	Growth	Absorption	Mucosa	IgA
<b>Strictly gluten free diet</b>				
L I 8 mo	+	NS	NS	-
S L P 1 y	+	NS	NS	+
A M P diet 2 1 y		NS	NS	+
R R 1 y 1 mo	+	NS	NS	+
K S 1 y 10 mo	+	NS	NS	-
M U 2 y 5 mo	+	+	±	-
H K 2 y 7 mo	+	+	-	Absent
E K 3 y	+	+	+	NS
H T 3 y 9 mo	-	+	-	+
T M 4 y 1 mo	-	+	-	NS
(J P) 3 y 5 mo <sup>d</sup>	+	±	±	NS
J P 5 y 10 mo	+	+	+	NS
<b>Poorly controlled gluten free diet</b>				
J F 2 y 1 mo	+	±	-	-
M L diet 2 2 y 7 mo		±	-	-
L O diet 2 3 y 4 mo	+	-	-	-
T K 3 y 7 mo	-	+	-	-
S E 3 y 10 mo	+	+	+	-
<b>Gluten free + unrestricted diet</b>				
M L diet 1 1 y 0 mo + 2 y 4 mo		NS	NS	NS
A H 2 y 0 mo + 0 y 4 mo	+	-	-	-
E K 2 y 0 mo + 0 y 6 mo	+	-	-	-
H N 5 y 5 mo + 0 y 1 mo		+	-	NS
A M P diet 1 3 y 2 mo + 2 y 1 mo	+	-	-	NS
L O diet 1 0 y 6 mo + 2 y 3 mo	+	-	-	NS
M H 3 y 0 mo + 0 y 7 mo	+	-	-	+

<sup>a</sup> Explanations of symbols in table + = full normalisation ± = improvement - = no change NS = not studied

<sup>b</sup> Initial biopsy failed

<sup>c</sup> Hypothyroid patient treated with thyroxine

<sup>d</sup> The patient J P was investigated twice after periods of different lengths

M U and S E in Table 5) with late onset of the disease no typical coeliac symptoms and severe iron-deficiency anaemia were initially treated with iron alone for 3 years and 6 months respectively and subsequently re-examined. No improvement in absorption function or mucosal damage was observed.

Treatment with gluten free diet (rice and corn being the only cereals allowed) was then started in every case. Table 5 presents the results obtained. Follow up examinations were made in 20 cases; however in 1 patient with hypothyroidism induced by thyroiditis (M H) no conclusion could be drawn in view of the impracticability of distinguishing the effects of the gluten free diet and the thyroiditis given simultaneously. In others (19 cases) a good clinical response especially in regard to growth was found in 17 cases, improvement in absorptive function in 11 of the 16 cases tested and improvement in the intestinal mucosa in 3 cases out of 13. Some response in respect of either growth, absorptive function or mucosa was found in all 19 cases.

The patients on a strictly gluten free diet invariably displayed a clinical response, nor malabsorption of the absorptive function and in some even normalisation of the mucosa although not until treatment had been continued for 2-5 years. Serum IgA showed a decrease in many patients. Precipitins to gluten disappeared in all but 1 case but precipitins to cow's milk persisted in 4 of the 10 patients who were initially found to have precipitins.

The patients on a poorly controlled gluten free diet although with restricted gluten were all also in good clinical condition, growing well and even exhibiting improvement in the absorptive function. The mucosal damage did not heal in this group and the serum IgA level remained unchanged. Precipitins to gluten were also found during treatment in 1 patient.

The third group includes cases in which the patients were initially on a gluten free and then on a more or less normal diet. All except 1 patient showed initially a response in growth but premature discontinuation of dietary treat-

Table 6 Development of serum IgA concentration and precipitins to cow's milk and to gluten when a relapse was developing in a patient (A M P)

The patient was initially treated with a poorly controlled gluten free diet for 3 years and 2 months with a rather good clinical response. Then 2 years 1 month before the start of a new diet period the patient was started on an unrestricted diet. A change in the growth pattern and anaemia were the only clinical symptoms. In the table the time of beginning a new gluten free diet is denoted by 0.

	Time			
	-2 y	-1 y	0	+5 mo
Serum IgA $\mu\text{g}/100 \text{ ml}$	22	90	146	16
Precipitins to cow's milk	+	+		-
Precipitins to gluten	-	-		-

ment arrested the progress towards recovery. The final results were dependent on the length of the period of gluten-containing diet. All 3 patients kept on a gluten-containing diet for more than 2 years suffered clinical relapse. The clinical relapses were manifested by a change in the growth pattern. In addition 1 patient had convulsions of unknown etiology (hypoglycaemia?). The serum IgA was high in all three during the relapse and precipitins were found. Two of the others displayed an increase in serum IgA and an increase in precipitins some months after the start of the unrestricted diet. In 1 case these immunological variants were followed when clinical relapse was developing (Table 6).

#### COMPARISON OF MANIFESTATIONS AND RESPONSES TO THERAPY IN PATIENTS OF DIFFERENT TYPES

It was found that the patients with diabetes mellitus and  $\alpha$ - $\gamma$ A globulinaemia all had some typical coeliac symptoms. Absorption tests revealed clear malabsorption except that the diabetics had a normal D-xylose test. The diabetics responded rather slowly to the diet, and normalisation of the mucosa was not observable in these patients but the diet was strict in only 1 patient out of 3. However the patient with

a  $\gamma$ A-globulinaemia responded completely to the gluten free diet

Among the patients without any significant concomitant disease two main groups can be distinguished. 11 patients had displayed gastrointestinal symptoms even during infancy (early onset group) and in 7 the onset occurred after 2 years of age (late onset group). In the former group gastrointestinal symptoms were rather common subsequently. Nevertheless three of these patients were later completely without gastrointestinal symptoms. As were also 6 of the 7 patients with late onset. Retardation of growth was more pronounced in the patients with early onset along with various nutritional defects, except for iron deficiency and megaloblastic anemia which were again commoner or more severe in the late onset group. The results of absorption tests were similar in the two groups. The 2 patients who primarily (without any previous therapy) had only partial villous atrophy belonged to the late onset group. No conclusions can be drawn in regard to the responses to therapy in the different types of disease.

## DISCUSSION

The first question which may arise from this report is whether these patients were suffering from coeliac disease. The symptomatology was certainly not classic in nature: some signs of malabsorption were discovered in every one and a favourable response to a gluten free diet was observed in all the 19 cases in which a proper follow up was possible, but the permanency of the gluten intolerance was not tested systematically. If this satisfies the criteria of coeliac disease then the patients may be termed coeliacs. The authors are of the opinion that they are all in fact coeliacs although it must be admitted that this was not completely confirmed.

The origin of diabetic enteropathies has often been discussed (4, 9). The chronological relationships of the diseases (Table 3), the findings and the responses to the diet suggest that in the

present series of patients the enteropathy is not a complication of diabetes, but more probably an independent disease, coeliac disease, which for some reason combines with diabetes more often than could be expected from chance alone. It should also be noted that diabetes was commoner in the sibs and other relatives of these non-diabetic patients than would be anticipated. The patient with a  $\gamma$ A globulinaemia had typical coeliac disease with confirmed gluten intolerance.

Gluten intolerance (coeliac disease) usually becomes manifest some months after the introduction of cereals into the diet. Consequently the diagnosis is usually made before the child reaches the age of 2 years. Why then was the diagnosis made so late in this group of patients? The different reasons for this can be enumerated as follows:

- 1 early onset, but spontaneous remission of symptoms for some years (9 patients)
- 2 early onset and continuous symptoms so slight that the diagnosis was postponed (1 patient)
- 3 early onset but remission with a brief and more or less incomplete trial with gluten free diet without any real diagnosis being made (5 patients)
- 4 late onset of the disease (7 patients)

From the practical standpoint it is very important to note that the actual symptomatology of patients in this age group is usually atypical: retardation of growth and anaemia often being the only symptoms. The retrospective data concerning stools are very unreliable. More over real diarrhoea is often lacking and the patients may even be constipated. Abdominal pain is another symptom worth noting. Nutritional defects are further important diagnostic indications: in fact we have found that coeliac disease is the commonest cause of severe iron deficiency, megaloblastic anemia and deficiency rickets in children over 2 years of age.

The results of absorption tests demonstrate that at least as far as those applied in this study are concerned no single one alone would pro-

the correct answer in all patients. This is in conformity with the observations of some of our research workers (6). Apparently the most reliable single test is that based upon cal fat. As the authors have suggested earlier (1) a considerably higher degree of reliability detecting malabsorption can be achieved by use of a multiple test pattern. However the aim of this report emphasizes the importance of biopsy for final diagnosis particularly in typical cases.

Very few conclusions can be drawn from the results of treatment. It is evident that maintenance of a strictly gluten free diet is extremely difficult in this age group and thus an objective method for checking observance of the diet is needed. The finding of precipitins to gluten practicable in many cases but unfortunately does not detect all. Marked elevation of the serum IgA level and reappearance of precipitins herald a clinical relapse.

The results of treatment demonstrate that restriction of gluten leads to clinical improvement and better absorptive function but total exclusion of gluten is necessary for normalisation of the mucosa which in any event is a very low process. In every case in this series re-imperson of a gluten-containing diet even if somewhat restricted induced a clinical relapse after some years.

### SUMMARY

This paper reports on the clinical picture and findings in 22 children over 2 years of age in whom the malabsorption syndrome was suspected and significant intestinal villous atrophy was found. Gluten intolerance was demonstrated in every one of the 19 patients in whom the results of treatment were followed. The authors consider that all the patients of this series were suffering from coeliac disease. Three of the 22 patients had concomitant diabetes mellitus and one of these additionally autoimmune thyroiditis. One patient had  $\alpha$ - $\gamma$  globulinemia. The correlations of these diseases are discussed.

Although the diagnoses in these cases were

made when the patients were over 2 years old in 15 cases the first symptoms had been seen during infancy. The main reasons for the postponement of diagnosis were spontaneous remission of the usual symptoms, amelioration of the symptoms on treatment and in some cases actual late onset of the disease. The classic clinical picture of coeliac disease was rare and in many cases especially those in which the symptoms started late the condition was only manifested by a few symptoms, retarded growth and anaemia. The results of absorption tests indicated that no single test is absolutely reliable for detecting malabsorption but that application of multiple tests increases the reliability. The laboratory tests revealed many nutritional defects the commonest of which was iron deficiency. Stress is laid upon the importance of biopsy for confirming the diagnosis. The results of treatment demonstrate that the restriction of gluten induces clinical and absorptive improvement but normalisation of the villous structure was achieved only by strict elimination over a period of many years if at all. Patients with a relatively or totally unrestricted diet all relapsed after some years. Elevation of the serum IgA level and reappearance of precipitins preceded the relapse.

### REFERENCES

1. Buchan D J & Gerrard J W. Coeliac disease: Problems in diagnosis and effects of a gluten free diet. *Ann Intern Med* 57: 85 1964.
2. Gerrard J W & Lubon M C. The malabsorption syndromes. *Pediatr Clin N Amer* 14: 75 1967.
3. Hamilton J R, Lynch M J & Reilly B J. Active coeliac disease in childhood. *Quart J Med* 38: 135 1969.
4. Elford C, Dewar M, Kretschmar & Dainton J. Malabsorption and diabetes mellitus in children. *Hist Pathol Acta* 23: 478 1963.
5. Kamenetz M. Duodenoscopy in malabsorption syndromes in infants. *Ann Pediatr Fenn* 12: 101 1966.
6. McFarlane A S & Willoughby M I. N. Who blood folate as a screening test for coeliac disease in childhood. *Lancet* 1: 442 1969.
7. Newman J L, Oak F K, Diamond C & Varrier C F & Schwartzman H. The gastrointestinal effects of iron deficiency anemia. *Pediatrics* 33: 85 1964.

a- $\gamma$ A globulinaemia responded completely to the gluten free diet

Among the patients without any significant concomitant disease two main groups can be distinguished. 11 patients had displayed gastrointestinal symptoms even during infancy (early onset group) and in 7 the onset occurred after 2 years of age (late onset group). In the former group gastrointestinal symptoms were rather common subsequently. Nevertheless three of these patients were later completely without gastrointestinal symptoms as were also 6 of the 7 patients with late onset. Retardation of growth was more pronounced in the patients with early onset along with various nutritional defects, except for iron deficiency and megaloblastic anaemia which were again commoner or more severe in the late onset group. The results of absorption tests were similar in the two groups. The 2 patients who primarily (without any previous therapy) had only partial villous atrophy belonged to the late-onset group. No conclusions can be drawn in regard to the responses to therapy in the different types of disease.

## DISCUSSION

The first question which may arise from this report is whether these patients were suffering from coeliac disease. The symptomatology was certainly not classic in nature, some signs of malabsorption were discovered in every one and a favourable response to a gluten free diet was observed in all the 19 cases in which a proper follow up was possible but the permanency of the gluten intolerance was not tested systematically. If this satisfies the criteria of coeliac disease then the patients may be termed coeliacs. The authors are of the opinion that they are all in fact coeliacs although it must be admitted that this was not completely confirmed.

The origin of diabetic enteropathies has often been discussed (4-9). The chronological relationships of the diseases (Table 3), the findings and the responses to the diet suggest that in the

present series of patients the enteropathy is not a complication of diabetes, but more probably an independent disease, coeliac disease, which for some reason combines with diabetes more often than could be expected from chance alone. It should also be noted that diabetes is commoner in the sibs and other relatives of these non-diabetic patients than would be anticipated. The patient with a  $\gamma$ A globulinaemia had typical coeliac disease with confirmed gluten intolerance.

Gluten intolerance (coeliac disease) usually becomes manifest some months after the introduction of cereals into the diet. Consequently the diagnosis is usually made before the child reaches the age of 2 years. Why, then, was the diagnosis made so late in this group of patients? The different reasons for this can be enumerated as follows:

- 1 early onset but spontaneous remission of symptoms for some years (9 patients)
- 2 early onset and continuous symptoms so slight that the diagnosis was postponed (1 patient)
- 3 early onset, but remission with a brief and more or less incomplete trial with gluten free diet without any real diagnosis being made (5 patients)
- 4 late onset of the disease (7 patients)

From the practical standpoint it is very important to note that the actual symptomatology of patients in this age group is usually atypical: retardation of growth and anaemia often being the only symptoms. The retrospective data concerning stools are very unreliable. More over real diarrhoea is often lacking and the patients may even be constipated. Abdominal pain is another symptom worth noting. Nutritional defects are further important diagnostic indications. In fact we have found that coeliac disease is the commonest cause of severe iron deficiency, megaloblastic anaemia and deficiency rickets in children over 2 years of age.

The results of absorption tests demonstrate that at least as far as those applied in this study are concerned no single one alone would pro-

## FIBRINOGEN AND FIBRINOLYSIS IN THE RESPIRATORY DISTRESS SYNDROME OBSERVATION DURING THE FIRST DAY OF LIFE

B KARITZKY W FRINGSHEIM and W KONZER

From the Children's Hospital University of Freiburg (FR Western Germany)

The relationship between the idiopathic respiratory distress syndrome (IRDS) of premature and blood coagulation has been the subject of discussion for some time. Particular attention has been paid to fibrinogen and fibrinolysis (1-10, 11-15). The situation is complicated by the fact that in almost three quarters of the cases that come to autopsy cerebral haemorrhage usually in the region of a choroid vessel is a major finding (4).

It would doubtless be pertinent to ascertain whether cerebral haemorrhage is of primary significance in these cases and possibly elicits symptoms which cannot be differentiated from those in genuine IRDS or even causes the full syndrome including the pulmonary changes or whether alternatively the hypoxia and hypercapnia of IRDS precipitate cerebral haemorrhage in these high risk infants (3). In the latter case discrimination between a loss of coagulation potential sequential to hepatic hypoxia (12) and intravascular coagulation (16) would be essential.

In the present study immunological methods requiring very small blood samples were applied thus allowing serial assays of plasma fibrinogen and spontaneous *in vitro* fibrinolysis during the first 24 hours of life. The examinations were carried out on normal premature infants and infants with IRDS of varying severity. The results were analysed statistically.<sup>1</sup>

Our thanks are due to Dipl. Math. Pfander Institut für Medizinische Statistik und Dokumentation der Universität Freiburg.

## MATERIALS AND METHODS

## Plasma Samples

48 healthy premature infants with birth weights between 1100 g and 2500 g none of whom had signs of respiratory distress or died were assigned to the normal collective. A second group comprised 18 premature infants with birth weights between 1190 g and 2280 g who presented the clinical signs of IRDS (high respiratory rate, retractions, grunting, cyanosis, asphyxia and a typical chest X-ray) and recovered. Finally fibrinogen and spontaneous fibrinolysis were determined in plasma from premature infants who died in the course of severe IRDS, all of whom had hyaline membranes. These were divided into two groups. The first contained 11 infants with birth weights between 870 g and 2450 g who had no cerebral haemorrhage at autopsy (the second 13 infants with massive cerebral haemorrhage at autopsy and 5 with sanguinolent liquor at postmortal puncture of the cisterna cerebromedullaris (=18 infants with birth weights between 1135 g and 2000 g). Six premature infants with birth weights between 1150 g and 1810 g dying of other causes such as oesophageal atresia, septicaemia and cardiac malformations were neither assigned to these groups nor considered in the statistical analysis. The plasma fibrinogen concentrations of these infants followed the typical rising course seen in normal infants during the first 24 hours of life (7).

The following four groups were compared by variance analysis and partly by multiple *t*-tests:

- 1 Normal premature infants
- 2 Premature infants with IRDS surviving
- 3 Premature infants dying of IRDS without cerebral haemorrhage
- 4 Premature infants dying of IRDS with cerebral haemorrhage

Table 1 shows the number of premature infants in the various groups with weight ranges and mean values.

The therapy of IRDS consisted of incubator care at high ambient oxygen concentrations and in the infusion of glucose solutions containing sodium bicarbonate for correction of acidosis according to serial



- 8 Nilsson L R, Soderhjelm L, Hoglund N J & Tornblom N. Infantulism hos spruceptent utan tarmsvår. *Nord Med* 60 1317 1958
- 9 Vinnik I E, Kern F Jr & Struthers J E Jr. Malabsorption and the diarrhea of diabetes mellitus. *Gastroenterology* 43 210 1962
- 10 Visakorpi J K, Immonen P & Kuitunen P. Malabsorption syndrome in childhood. The occurrence of absorption defects and their clinical significance. *Acta Paediat Scand* 56 1 1967
- 11 Visakorpi J K & Immonen P. Intolerance to cow's milk and wheat gluten in the primary mal-

absorption syndrome in infancy. *Acta Paediat Scand* 56 49 1967

Submitted Sept 8 1969

Accepted Nov 4 1969

(J. K. V.) Children's Hospital  
Stenbackinkatu 11  
Helsinki 29  
Finland

**Key words:** Coeliac disease, malabsorption, gluten free diet, diabetic enteropathy,  $\alpha$ - $\gamma$ -A globulinaemia

Table 2 Variance analysis for fibrinogen concentrations measured at different hours of age

Age in hours		ms	df	F	Significance
1	Between groups	9041.28	3	3.37	—
	Error	2679.28	18		
2	Between groups	1676.59	3	0.47	—
	Error	3554.18	18		
6	Between groups	34674.60	3	8.93	—
	Error	3881.13	72		
12	Between groups	19039.15	3	4.07	—
	Error	4676.71	55		
24	Between groups	10401.81	3	1.88	—
	Error	5539.46	65		

ms = mean square df = degrees of freedom

the fibrinogen determinations at various hours of life. As there were no differences between groups at the ages of 2 and 24 hours *t* tests were not carried out (see statistical methods). The fibrinogen values—means and 95% confidence limits—are shown in Fig. 1 for each group at each age. The statistical evaluations allow the following conclusions to be drawn.

1. No clear differences in plasma fibrinogen concentrations between normal premature infants (Group 1) and infants with IRDS who survived (Group 2) can be found in any of the

age groups examined. The small difference at the age of one hour cannot be proved statistically.

Table 3 Multiple *t* test for the fibrinogen concentrations measured at 1, 6 and 12 hours of age

Age in hours	Group	Index	<i>t</i> value	Significance
1	1	2	2.06	—
		3	1.38	—
		4	3.02	—
		3	0.08	—
	2	4	1.34	—
		3	0.88	—
		1, 2, 3, 4	1.91	—
6	1	2	0.36	—
		3	1.08	—
		4	4.87	—
		3	1.21	—
	2	4	4.36	—
		3	2.31	—
		1, 2, 3, 4	3.73	—
12	1	2	0.28	—
		3	0.43	—
		4	3.45	—
		3	0.21	—
	2	4	2.91	—
		3	2.24	—
		1, 2, 3, 4	2.40	—

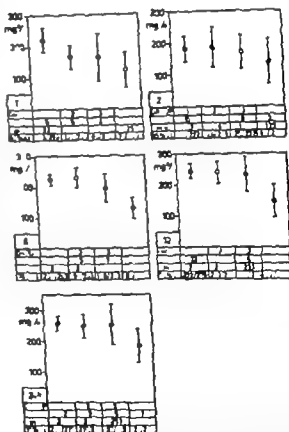


Fig. 1 Mean values and 95% confidence limits for plasma fibrinogen concentrations of premature infants at 1, 2, 6, 12 and 24 hours of age. See text for definition of groups. *n* = number of infants examined; *mv* = mean value; 95% *cl* = 95% confidence limit.

Table 1 Number of premature infants in the various groups with weight ranges and mean values

Group	1	2	3	4
<i>n</i>	48	19	11	11
<i>Birth weights</i>				
Mean values	1900 g	1890 g	1480 g	1360 g
Ranges	1100-2500 g	1190-2280 g	870-2450 g	1135-2000 g

micro Astrup measurements. Blood or plasma were not given in the cases examined.

The samples consisted of 1.4 ml blood collected into 3.8 sodium citrate in the relationship 10:1 partly from venepuncture with cannulae free of clots partly from indwelling umbilical venous catheters. No inhibitors of fibrinolysis were used. Sampling was carried out immediately after arrival of the infant on the premature ward and at the age of 6, 12 and 24 hours. After subsequent aspiration from the erythrocytes the plasma was frozen in all cases until the assay was undertaken.

#### Antisera

Only monospecific antisera from rabbits were used which react solely with human fibrinogen and its split products (5).

#### Immunological assay of fibrinogen and fibrinolysis

Fibrinogen determinations were carried out by a modification of the radial immunodiffusion method (9). As fibrinogen split products can appear in the sera of normal premature infants (2) all samples were recalcified and split product determinations carried out on the sera. The results of the fibrinogen measurements and those of the fibrinolysis assays were corrected accordingly.

Fibrinolysis was determined by a new semi quantitative immunological method (6). All determinations were done in triplicate and the mean values used for the statistical analyses. In our publication of 1968 the degree of fibrinolysis was expressed in terms of percentage of original fibrinogen. As this definition is not suitable for statistical methods the degree of fibrinolysis is now defined as the amount (in mg/100 ml) of antiserum positive substance which remains in the serum after recalcification of the plasma and incubation at 37°C for 24 hours. The expression in mg/100 ml is not quite correct as the substance set free from the clot during fibrinolysis has a lower molecular weight than fibrinogen (13). This difference is negligible however for the purpose of comparison between the four groups. The unit mg is therefore retained for fibrinolysis.

#### Statistics

Most of the infants were from 3 to 5 hours old at the time of admission so that these individuals first appear in the analysis at 6 hours of age. As a consequence there are relatively few values for the first

two hours of life. Only 12 infants died of illness, lived longer than 24 hours and a few measurements were marred by technical difficulties. Venepunctures were occasionally impossible during shock and some of the plasma samples clotting spontaneously at thawing. As it was therefore not possible to obtain values for all the infants at regular intervals separate statistical evaluations for each timepoint of sample were necessary. This means that for statistical purposes different groups were used for each sample timepoint. Comparison for error within groups therefore not strictly feasible.

The values for a time sample (= age in hours) were examined by simple variance analysis using groups listed in the section Plasma samples. If difference between the groups was significant (probability < 0.05) further comparison of groups by multiple *t* test ensued. Not only every possible pair but also Groups 1 and 2 (survivors) and 3 and 4 (dead) were compared.

In the tables showing variance analyses and *t* tests the following key is used:

- ~ not significant
- \* poor significance
- \*\* significant
- highly significant

#### Apparatus and instruments

The Eppendorf Microtiter System comprising reaction tubes, microburettes, centrifuge and thermostat was used throughout. Spontaneous fibrinolysis of recalcified plasma samples was allowed to take place in the polyethylene reaction tubes of this system at 37°C in the thermostatic cell. As the ports come into contact with the material under examination hydrophobic the system is particularly suitable for coagulation studies with small quantities of plasma.

The variance analyses were calculated on the IB 7040 computer in the University Dept. of Mathematics, Freiburg.

## RESULTS

#### Fibrinogen

Table 2 shows the results of the variance analyses. Table 3 those of the multiple *t* tests.

Eppendorf Gerätebau D 2 Hamburg 63, Germany.

1 Differences cannot be proved between the groups at 1 and 2 hours. Spontaneous fibrinolysis is equally pronounced and the values are higher than those found in adults (6).

2 At 6 hours significant differences can be found between the Groups 1 and 3 as well as 1 and 4. Fibrinolytic activity is significantly lower in all infants who died (Groups 3 and 4) as compared with normal infants and survivors from IRDS (Groups 1 and 2).

3 At 12 hours the results are comparable. Further differences are apparent between Groups 3 and 2 and 4 and 2. Those groups containing the infants who died (3 and 4) have highly significantly lower values for spontaneous fibrinolysis *in vitro* than those containing the survivors (1 and 2).

4 At 24 hours spontaneous clot lysis is hardly demonstrable for infants who died of IRDS whether they had cerebral haemorrhage (group 4) or not (group 3). Fibrin split products equivalent only to an average of 5 and 11  $\mu\text{g}/100\text{ ml}$  plasma were found after incubation of the clot for 24 hours at 37°C. In these groups fibrinolysis is significantly weaker (Group 3) and poorly significantly weaker (Group 4) than in the control group (1). If all survivors are taken as a group and compared with those who died significantly higher values for spontaneous fibrinolysis are found in *in vitro* plasmas. Fibrinolytic activity is relatively uniform in the survivor groups (1 and 2) during the first 24 hours of life. This is not the case for those infants who died of IRDS. At the time of entry fibrinolytic activity is equal to that in the control group. Later a continuous fall in activity ensues during the observation period of 24 hours. This trend cannot be tested statistically, however.

## DISCUSSION

### Fibrinogen

The results show that at no time during the first 24 hours of life statistically significant differences in plasma fibrinogen levels can be found between normal premature infants and

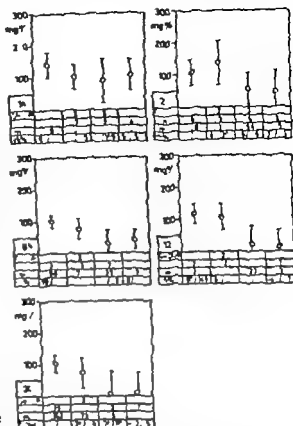


Fig 3 Mean values and 95% confidence limits for *in vitro* plasma spontaneous fibrinolysis of premature infants at 1, 2, 6, 12 and 24 hours of age. See text for definition of groups.  $n$  = number of infants examined.  $\bar{m}$  = mean value. 95%  $cl$  = 95% confidence limit. (Negative values for confidence limits are not as hatched in the diagram.)

such with the idiopathic respiratory distress syndrome who survive.

Only at the age of 1 hour is the mean value for survivors from IRDS (Group 2 = 168  $\text{mg}/100\text{ ml}$  plasma) lower than for the control group (1 = 221  $\text{mg}/100\text{ ml}$ ). However this difference cannot be proved statistically on account of the large degree of variance.

Later the values for uncomplicated IRDS (Group 2) are partially even higher than the controls (Group 1). Uncomplicated IRDS is therefore not accompanied by hypofibrinogenemia as assumed in the hypothesis of Stark *et al* (16). Postnatally no fall in plasma fibrinogen can be found in uncomplicated IRDS (Group 2) during the first 24 hours of life.

Table 4 Variance analysis for spontaneous fibrinolysis measured at different hours of age

Age in hours		ms	df	F	Significance
1	Between groups	2101.22	3	0.98	—
	Error	2133.97	15		
2	Between groups	7464.12	3	2.36	—
	Error	3165.82	17		
6	Between groups	16891.19	3	5.18	**
	Error	3263.51	64		
12	Between groups	31253.29	3	6.23	***
	Error	5014.17	45		
24	Between groups	25970.53	3	3.72	
	Error	6977.78	56		

ms = mean square df = degrees of freedom

2 In all age groups only slightly lower fibrinogen concentrations were found in Group 3 (IRDS died no cerebral haemorrhage) as compared with Group 1. In no case was the difference of statistical significance.

3 Only comparison of Group 4 (IRDS died cerebral haemorrhage) with the other three groups results in clear cut differences. At the age of 1 and 12 hours the fibrinogen concentrations are significantly lower than those of the normal controls (Group 1  $p < 0.05$ ) at the age of 6 hours this difference is highly significant ( $p < 0.01$ ). There are also differences between Groups 2 and 3 as compared with Group 4, some highly significant at the ages of 6 and 12 hours. As more than half the infants in Group 4 died before attaining the 24th hour of life the differences found particularly as compared with Group 1 cannot be proved statistically.

4 When the values for all of the survivors are compared as a group with the dead, highly significant differences are found at 6 hours, significant differences at 12 hours. Here as in 1969 (7) a continuous rise in plasma fibrinogen level during the first 24 hours of life is apparent. This rise took place in all four groups examined. The trend is obvious but cannot be tested statistically as the populations were not identical at the time of sampling (see statistical methods). Particularly in Group 4 those prematures with low fibrinogen values tended to die before the 24th hour so that the relative

ely high overall 24 hours value could in part be due to the elimination of these low values.

### Fibrinolysis

Table 4 shows the results of the variance analyses. Table 5 shows those for the  $t$  tests for fibrinolysis. The mean values and 95% confidence limits for the various age groups are shown in Fig. 2. Negative values for confidence are not included. Differences were found by variance analysis between the age groups 6, 12 and 24 hours. Only in these cases were  $t$  tests applied. The results are as follows:

Table 5 Multiple  $t$  test for spontaneous fibrinolysis measured at 6, 12 and 24 hours of age

Age in hours	Group index		$t$ value	Significance
6	1	2	1.13	—
	1	3	2.87	—
	1	4	3.27	—
	2	3	1.76	—
	2	4	1.73	—
	3	4	0.34	—
12	1+2	3+4	3.29	*
	1	2	0.44	—
	1	3	2.99	**
	1	4	3.51	—
	2	3	2.41	—
	2	4	2.84	—
24	3	4	0.26	—
	1+2	3+4	4.12	*
	1	2	1.07	—
	1	3	2.43	—
	1	4	2.61	*
	2	3	1.55	—
	2	4	1.57	—
	3	4	0.14	—
	1+2	3+4	2.83	—

1 Differences cannot be proved between the groups at 1 and 2 hours. Spontaneous fibrinolysis is equally pronounced and the values are higher than those found in adults (6).

2 At 6 hours significant differences can be found between the Groups 1 and 3 as well as 1 and 4. Fibrinolytic activity is significantly lower in all infants who died (Groups 3 and 4) as compared with normal infants and survivors from IRDS (Groups 1 and 2).

3 At 12 hours the results are comparable. Further differences are apparent between Groups 3 and 2 and 4 and 2. Those groups containing the infants who died (3 and 4) have highly significantly lower values for spontaneous fibrinolysis *in vitro* than those containing the survivors (1 and 2).

4 At 24 hours spontaneous clot lysis is hardly demonstrable for infants who died of IRDS whether they had cerebral haemorrhage (group 4) or not (group 3). Fibrin split products equivalent only to an average of 5 and 11 mg/100 ml plasma were found after incubation of the clot for 24 hours at 37°C. In these groups fibrinolysis is significantly weaker (Group 3) and poorly significantly weaker (Group 4) than in the control group (1). If all survivors are taken as a group and compared with those who died significantly higher values for spontaneous fibrinolysis are found in survivor plasmas. Fibrinolytic activity is relatively uniform in the survivor groups (1 and 2) during the first 24 hours of life. This is not the case for those infants who died of IRDS. At the time of entry fibrinolytic activity is equal to that in the control group. Later a continuous fall in activity ensues during the observation period of 24 hours. This trend cannot be tested statistically, however.

## DISCUSSION

### Fibrinogen

The results show that at no time during the first 24 hours of life statistically significant differences in plasma fibrinogen levels can be found between normal premature infants and

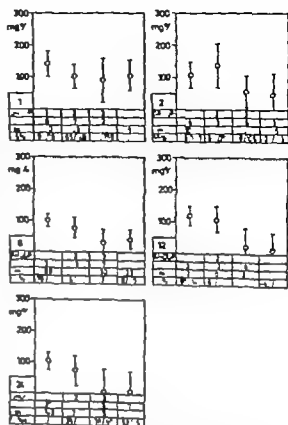


Fig. 2 Mean values and 95% confidence limits for *in vitro* plasma spontaneous fibrinolysis of premature infants at 1, 2, 6, 12 and 24 hours of age. See text for definition of groups. n = number of infants examined; mv = mean value; 95% ci = 95% confidence limit. (Negative values for confidence limits are not included in the diagrams.)

such with the idiopathic respiratory distress syndrome who survive.

Only at the age of 1 hour is the mean value for survivors from IRDS (Group 2 = 168 mg/100 ml plasma) lower than for the control group (1 = 221 mg/100 ml). However this difference cannot be proved statistically on account of the large degree of variance.

Later the values for uncomplicated IRDS (Group 2) are partially even higher than the controls (Group 1). Uncomplicated IRDS is therefore not accompanied by hypofibrinogen aemia as assumed in the hypothesis of Stark *et al.* (16). Potentially no fall in plasma fibrinogen can be found in uncomplicated IRDS (Group 2) during the first 24 hours of life.

On the contrary the mean value continues to rise during this period from 168 mg/100 ml to 248 mg/100 ml plasma. Assuming fibrinogen consumption would entail assuming rapid fibrinogen production by the liver to compensate not only for the losses into the hyaline membranes of the lungs but also for the intravascular losses during hypothetical disseminated intravascular coagulation.

The mean fibrinogen values in infants dying of IRDS without cerebral haemorrhage (Group 3) are lower than those in the control group in all assay periods. In this group a rise in mean fibrinogen concentration during the first day of life is also apparent (Fig 1). All of these infants had hyaline membranes at autopsy. This means that fibrinogen losses into the lungs are counteracted by increased fibrinogen synthesis in the liver. Moreover other organs susceptible to fibrinogen deposition during intravascular coagulation were not examined histologically.

The plasma fibrinogen concentrations of infants dying of IRDS with cerebral haemorrhage (Group 4) are already significantly lower than those in the control group at the age of one hour. At 6 and at 12 hours of age the mean value for group 4 is significantly lower than all three remaining groups. The difference at 24 hours could not be proved statistically because the low values in infants in group 4 dying earlier had been eliminated.

As the half life of adult fibrinogen is three days the low values in group 4 cannot be explained purely by postnatal hypoxia with subsequent insufficiency in liver synthesis. Loss of fibrinogen from the circulating plasma either during labour during the first hour of life or possibly even in utero must be assumed. Complete cessation of fibrinogen production in the liver is highly improbable as the plasma concentration continues to increase in spite of postnatal loss into hyaline membranes. This fact cannot be explained alone by release of fibrinogen from hypothetical depots. Intravascular coagulation as a cause of hypofibrinogenemia would have to have initiated and

presumably also ceased, before the first hour of life.

We have no explanation for these low fibrinogen values from the first hour of life onwards in infants dying of IRDS with cerebral haemorrhage as compared with those dying without cerebral haemorrhage. Are the fibrinogen values in the former low enough to cause haemorrhage? The mean values of this group were never lower than 100 mg/100 ml plasma, that value considered critical in the adult. Experimental work with newborn rabbits (8) and neuropathological findings (14) suggest that hypoxia can cause cerebral haemorrhage. Possibly relative hypofibrinogenemia in the hypoxic and hypercapnic infants of group 4 sufficed to precipitate cerebral haemorrhage.

### *Fibrinolysis*

The fibrinolytic activity in plasma of healthy premature infants and survivors from IRDS remains relatively constant during the first 24 hours of life. Furthermore at no time could significant differences between normal controls (Group 1) and survivors from IRDS (Group 2) be found. Spontaneous fibrinolysis in infants dying of IRDS (Groups 3 and 4) did not differ from that in the other two groups at the age of one hour. Later however from the 6th hour of life onwards significantly lower values were found in infants dying than in survivors. The fibrin clots from infants dying (Groups 3 and 4) were practically unlysable at 12 and 24 hours. These findings of premortal loss of fibrinolytic activity compare well with those published by Markarian *et al* (10) who used the euglobulin lysis method.

This fall in fibrinolytic activity *in vitro* in Groups 3 and 4 could be caused by consumption or inactivation of fibrinolytic potential. As the lower plasminogen concentrations found in these groups do not fall further during the first 24 hours of life as preliminary results show alterations of plasminogen activator systems or the influence of antiplasmin rather than a loss of plasminogen must be assumed.

Quantitative analysis of fibrin and fibrino-

gen split products in fresh serum did not elicit data the state of the fibrinolytic system *in vivo*. Split products were found in concentrations surpassing 8 mg/100 ml in the sera of 2 infants from Group 4, 2 infants from Group 3 and 1 infant from Group 2 but also in the sera of 8 healthy premature infants. Thus no correlation between the severity of IRDS and presence of fibrin and fibrinogen split products could be construed. Bonfacci *et al* (2) also found split products in cord blood of healthy newborn infants. The estimation of split products therefore has no prognostic value.

### SUMMARY

Serial assays of plasma fibrinogen and fibrinolysis in normal premature infants and premature infants with the Idiopathic Respiratory Distress Syndrome (IRDS) were made during the first day of life.

During the first 24 hours of life there were no significant differences in fibrinogen concentration between infants with uncomplicated IRDS and normal infants. In those infants who died of IRDS without cerebral haemorrhage slightly lower fibrinogen values were found. Premature infants who died of IRDS with cerebral haemorrhage had lower fibrinogen values than those in the other three groups from the first hour of life onwards. The plasma fibrinogen concentration rose during the first 24 hours of life in all four groups. The degree of spontaneous fibrinolysis *in vitro* was equal in all four groups at the second and sixth hour of life. Fibrinolysis subsequently fell to hardly measurable values in infants who died whereas it remained relatively constant in normal premature infants and survivors from IRDS.

### REFERENCES

- Andres C M, Wauters D H, Murphy D, Dowd J E, Nusselder K E, & Ambrose J L. The fibrinolytic system in pulmonary and other diseases. *J Clin Pathol* 12: 10 (1963).
- Bonfacci E, Rizzo P, & Gravina E. Demonstration of split products of fibrinogen in the blood of normal newborns. *Ital Neonat* 12: 28 (1968).
- Harrison V C, Hesse H, & Jelen M. Intracranial haemorrhage associated with hyaline membrane disease. *Archiv Dis Child* 43: 116 (1968).
- Kabat L, Schenck W, & Kuster W. Atemnotsyndrom und Blutgerinnungsaktivität. *Z Kinderheilk* 105: 55 (1969).
- Kanitzky D, & Kuster W. Herstellung eines immunogenischen Antiserums gegen menschliches Fibrinogen. *Thrombos Diathes Haemorrh* 18: 532 (1967).
- Eine semiquantitative immunologische Methode zur Fibrinogenbestimmung. *Thrombos Diathes Haemorrh* 19: 533 (1968).
- Kanitzky D, Pringheim W, Ziemer O, & Kuster W. Veränderungen der Fibrinogenkonzentration im Plasma gesunder Frühgeborener am ersten Lebenstag. *Z Kinderheilk* 105: 71 (1969).
- Ludwig H. Mikrozirkulationsstörungen und Druckeinstellungen im frühen Genuß bei Hypoxie. *Blutthromb Gynecol* (Basel) 46: 1968.
- Mancini G, Carbonara A O, & Heremans J F. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 2: 235 (1965).
- Markman M, Lindley A, Lebchenko L O, & Gibbes J H. Fibrinolytic activity in premature infants with the respiratory distress syndrome. *J Pediatr* 67: 957 (1965).
- Markman M, Gibbes J H, Jackson J J, Bamson A E, Lindley A, Rosenblatt E, Martorell R, & Lebchenko L O. Fibrinolytic activity in premature infants. *Amer J Dis Child* 113: 312 (1967).
- Markman M, Lindley A, Jackson J J, & Bamson A. Coagulation factors in pregnant women and premature infants with and without the respiratory distress syndrome. *Thrombos Diathes Haemorrh* 17: 385 (1967).
- Meunier J V, Delaplace M, & Gruber P. Les produits de dégradation du fibrinogène humain par le plasme. *Ann Inst Pasteur* 100: 490 (1961).
- Towbin A. Cerebral intraventricular hemorrhage and subependymal matrix infarction in the fetus and premature newborn. *Amer J Path* 52: 121 (1968).
- Schwartz E A, Cook C D, & Rudolph A J. Fibrinolytic activity in the serum of infants with and without the hyaline membrane syndrome. *Acta Paediatr Scand* 49: 727 (1960).
- Stark C B, Abrahamson D, & Erkan Y. Intravascular coagulation and hyaline membrane disease of the newborn. *Lancet* i: 1190 (1968).

Submitted Sept. 6 1969

Accepted Nov. 10 1969

(D. K.) Univ. Kinderklinik  
D 78 Freiburg i. Brg.  
Germany

Key words: Neonate, blood coagulation, in the newborn, fibrinogen, fibrinolysis, idiopathic respiratory distress syndrome.



On the contrary the mean value continues to rise during this period from 168 mg/100 ml to 248 mg/100 ml plasma. Assuming fibrinogen consumption would entail assuming rapid fibrinogen production by the liver to compensate not only for the losses into the hyaline membranes of the lungs but also for the intravascular losses during hypothetical disseminated intravascular coagulation.

The mean fibrinogen values in infants dying of IRDS without cerebral haemorrhage (Group 3) are lower than those in the control group in all assay periods. In this group a rise in mean fibrinogen concentration during the first day of life is also apparent (Fig. 1). All of these infants had hyaline membranes at autopsy. This means that fibrinogen losses into the lungs are counteracted by increased fibrinogen synthesis in the liver. Moreover other organs susceptible to fibrinogen deposition during intravascular coagulation were not examined histologically.

The plasma fibrinogen concentrations of infants dying of IRDS with cerebral haemorrhage (Group 4) are already significantly lower than those in the control group at the age of one hour. At 6 and at 12 hours of age the mean value for group 4 is significantly lower than all three remaining groups. The difference at 24 hours could not be proved statistically because the low values in infants in group 4 dying earlier had been eliminated.

As the half life of adult fibrinogen is three days the low values in group 4 cannot be explained purely by postnatal hypoxia with subsequent insufficiency in liver synthesis. Loss of fibrinogen from the circulating plasma either during labour during the first hour of life or possibly even in utero must be assumed. Complete cessation of fibrinogen production in the liver is highly improbable as the plasma concentration continues to increase in spite of postnatal loss into hyaline membranes. This fact cannot be explained alone by release of fibrinogen from hypothetical depots. Intravascular coagulation as a cause of hypofibrinogenemia would have to have initiated, and

presumably also ceased before the first hour of life.

We have no explanation for these low fibrinogen values from the first hour of life onwards in infants dying of IRDS with cerebral haemorrhage as compared with those dying without cerebral haemorrhage. Are the fibrinogen values in the former low enough to cause haemorrhage? The mean values of this group were never lower than 100 mg/100 ml plasma that value considered critical in the adult. Experimental work with newborn rabbits (8) and neuropathological findings (14) suggest that hypoxia can cause cerebral haemorrhage. Possibly relative hypofibrinogenemia in the hypoxic and hypercapnic infants of group 4 sufficed to precipitate cerebral haemorrhage.

#### *Fibrinolysis*

The fibrinolytic activity in plasma of healthy premature infants and survivors from IRDS remains relatively constant during the first 24 hours of life. Furthermore at no time could significant differences between normal controls (Group 1) and survivors from IRDS (Group 2) be found. Spontaneous fibrinolysis in infants dying of IRDS (Groups 3 and 4) did not differ from that in the other two groups at the age of one hour. Later however from the 6th hour of life onwards significantly lower values were found in infants dying than in survivors. The fibrin clots from infants dying (Groups 3 and 4) were practically unlysable at 12 and 24 hours. These findings of premortal loss of fibrinolytic activity compare well with those published by Markarian *et al* (10) who used the euglobulin lysis method.

This fall in fibrinolytic activity *in vitro* in Groups 3 and 4 could be caused by consumption or inactivation of fibrinolytic potential. As the lower plasminogen concentrations found in these groups do not fall further during the first 24 hours of life as preliminary results show alterations of plasminogen activator systems or the influence of antiplasmin rather than a loss of plasminogen must be assumed.

Quantitative analysis of fibrin and fibrino-

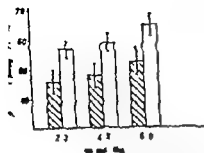


Fig. 1. Average values and range of phagocytosis of *E. coli* in various ages. Children with rickets in striped columns; controls in white columns.

hanced by Kabat & Mayer (7) was used to determine the 50% hemolytic units of complement activity (C.H. units).

**Bactericidal activity.** Bactericidal activity of leukocytes was evaluated by the method described by Müller & Becker (13).

**Immunoglobulin levels.** The evaluation of IgG and IgM levels was performed by means of the quantitative immunoprecipitation method on Pärtega plate (Behringwerke AG Marburg) according to Becker *et al.* (1).

**Statistical method.** The variables obtained were subjected to the  $\chi^2$  test and the probability was calculated.

## RESULTS

**Dependence of opsonins on the age and Ca  $\times$  P index.** Following the dependence of heat stable serum opsonizing activity on the age, the increase of their values with growth can be seen (Fig. 1). There are significant differences between rickets and controls ( $p < 0.005$ ). In addition the lowering of heat stable opsonin levels is connected with the severity of the illness expressed by Ca  $\times$  P index (Fig. 2).

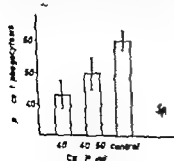


Fig. 2. Average values and range of phagocytosis of *E. coli* in groups with different Ca  $\times$  P indexes.

Table 1. Phagocytosis of *E. coli* in normal serum and serum in rickets by normal and rickets leukocytes

Source of serum	Source of leukocytes	
	Normal	Rickets
Normal	60%	63
Rickets	42%	46%

**Bactericidal activity.** The patient's leukocytes were equally as effective in killing of *E. coli* as were the leukocytes from normal controls. Mean number of viable bacteria after 1 hour has been  $2 \times 10^4$  in normal children and  $5 \times 10^4$  in rickets; after 2 hours  $1.5 \times 10^3$  and  $4 \times 10^3$  respectively. No significant differences were noted either in the rate of killing or in the number of viable bacteria at the end of the test period.

**Leukocytes and serum in rickets.** Serum and peripheral leukocytes from normal subjects and children with rickets were compared in the phagocytic tests which were performed in 5 cases (Table 1). As seen the serum was responsible for the variation in phagocytosis.

**Serum opsonizing activity.** The two groups of 7 children each were tested for serum opsonizing activity for *E. coli*. The mean phagocytosis in unheated serum was 56 per cent for children with rickets and 74 per cent for controls. Heated aliquots of the same serums resulted in phagocytosis of 41 and 58 per cent respectively. Thus the heat stable component of opsonin seems to be essential for diminished phagocytosis in rickets (Fig. 3).

**Complement activity.** Since it has been demonstrated that hemolytic complement may take part in the process of opsonization, complement activity was compared in the serums of the normal controls and those of children with rickets. The 50 per cent hemolytic units of complement are seen to be similar in these two groups (Fig. 3).

**Test for serum phagocytosis inhibitor.** In view of marked depression of phagocytosis in rickets an inhibitor was considered and tested

## EVALUATION OF PHAGOCYTOSIS IN RICKETS

J STRÖDER and P KASAL

From the Department of Paediatrics (Head J Stroder) University of Wurtzburg Germany

Vitamin D deficient rickets is conspicuously accompanied by infections (8). Here the problem arises, of whether these accompanying diseases are caused by an increased exposure to infection or by a certain insufficiency of the defense system of an organism.

Cases of rickets occur frequently in families of lower social level where there is an increased possibility of food contamination or inhalation infection. On the other hand however a certain damage to the defense system of the organism evoked by rickets may also cause the increased incidence of infections. This possibility is supported by the observation of a decreased cellular and humoral immunity in young rats with experimentally induced rickets (18). In an attempt to study this problem in children we carried out examinations of phagocytosis, bacteriotoxicity and of complement levels in infants with rickets. *Escherichia coli* was used in all experiments because children are usually exposed to this antigen in the early stages of life and subsequently produce antibodies against this bacteria (9, 10).

### MATERIAL AND METHODS

Studies were performed on 30 children with rickets verified by x-ray examination, biochemical findings and cranio-tables (6) (13 boys, 17 girls) and on 33 healthy children (15 boys, 18 girls). The children were divided into 3 age groups. Rickets groups: 2-3 months (9 cases), 4-5 months (14 cases), 6-8 months (7 cases); control groups: 2-3 months (12 cases), 4-5 months (12 cases), 6-8 months (9 cases). The determinations of calcium and phosphorus blood levels in all cases were performed and  $\text{Ca} \times \text{P}$  indexes

calculated (4). There were 9 children with  $\text{Ca} \times \text{P}$  index below 40 and 12 infants with this index between 40 and 50 in the rickets group.

**Blood.** The blood was obtained by venipuncture and collected in test tubes. Aliquots of 3 ml were placed in equal volumes of 6 per cent clinical dextran molecular weight 75 000 (Dr Fresenius Bad Homburg) in physiologic saline solution with 70 m heparin 135 000 I U/g (Werner Schrur Hamburg) per 100 ml.

**Preparation of leukocytes.** Aliquots of blood in dextran solution as described above were sedimented 1 hour at room temperature. The leukocyte rich supernatant was removed and washed three times in TC medium 199 with Earle's BSS (Serva Heidelberg) and 0.5 human serum albumin (Behringwerke AG Marburg). Amounts of  $1.25 \times 10^6$  leukocytes per ml were used within an hour of preparation.

**Preparation of serum.** Blood was drawn by venipuncture and allowed to clot at room temperature. Serums obtained were either heated at 56°C in a water bath for 30 min or used fresh without any additional treatment.

**Preparation of bacteria.** Bacterial suspensions were made from 24 hours old cultures of *Escherichia coli* B 2340 grown in CT medium (Bactotrypton 10 g, Yeast extract 1 g, NaCl 8 g ad 1000 ml H<sub>2</sub>O) with 1 mM of calcium chloride and 1% of glucose. The suspensions were washed twice with isotonic saline and resuspended after counting in Helber chamber so that 0.025 ml contained 25-10<sup>6</sup> bacteria.

**Phagocytic test.** The experimental procedure used in studying the phagocytic rate was essentially that of Winkelstein & Drachman (21). Aliquots of leukocyte suspension containing  $12.5 \times 10^6$  cells per ml were placed in pyrex tubes. After centrifugation at 900 rpm for five minutes the supernatant was removed. Bacteria in the amount of  $25 \times 10^6$  in 0.025 ml were added as well as 0.5 ml of either heated or unheated serum diluted 1:10 in TC medium 199. After gentle mixing the tubes were sealed for 30 min and then stained with methylene blue. The percentage of phagocytosis was determined by counting of 300 leukocytes and scoring of those with ingested bacteria.

**Hemolytic assay of complement.** The method out

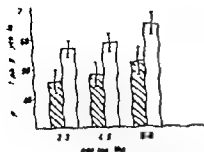


Fig. 1. Average values and range of phagocytosis of *E. coli* at various ages. Children with rickets in striped columns; controls in white columns.

hood by Kabat & Mayer (7) was used to determine the 50% hemolytic units of complement activity (CH units).

**Bactericidal activity.** Bactericidal activity of leukocytes was evaluated by the method described by Miller & Backler (13).

**Immunoglobulin levels.** The evaluation of IgG and IgM levels was performed by means of the quantitative immunoprecipitation method on Partigen plate (Behringwerke AG Marburg) according to Becker *et al.* (1).

**Statistical method.** The variables obtained were subjected to the *t* test and the probability was calculated.

## RESULTS

**Dependence of opsonins on the age and Ca  $\times$  P index.** Following the dependence of heat stable serum opsonizing activity on the age, the increase of their values with growth can be seen (Fig. 1). There are significant differences between rickets and controls ( $p < 0.005$ ). In addition, the lowering of heat stable opsonin levels is connected with the severity of the illness, expressed by Ca  $\times$  P index (Fig. 2).

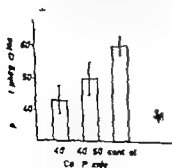


Fig. 2. Average values and range of phagocytosis of *E. coli* in groups with different Ca  $\times$  P indexes.

Table 1. Phagocytosis of *E. coli* in normal serum and serum in rickets by normal and rickets leukocytes

Source of serum	Source of leukocytes	
	Normal	Rickets
Normal	60%	63%
Rickets	42%	46%

**Bactericidal activity.** The patients' leukocytes were equally as effective in killing of *E. coli* as were the leukocytes from normal controls. Mean number of viable bacteria after 1 hour has been  $2 \times 10^4$  in normal children and  $5 \times 10^4$  in rickets; after 2 hours  $1.5 \times 10^3$  and  $4 \times 10^3$  respectively. No significant differences were noted either in the rate of killing or in the number of viable bacteria at the end of the test period.

**Leukocytes and serum in rickets.** Serum and peripheral leukocytes from normal subjects and children with rickets were compared in the phagocytic tests, which were performed in 5 cases (Table 1). As seen, the serum was responsible for the variation in phagocytosis.

**Serum opsonizing activity.** The two groups of 7 children each were tested for serum opsonizing activity for *E. coli*. The mean phagocytosis in unheated serum was 56 per cent for children with rickets and 74 per cent for controls. Heated aliquots of the same serums resulted in phagocytosis of 41 and 58 per cent respectively. Thus the heat stable component of opsonin seems to be essential for diminished phagocytosis in rickets (Fig. 3).

**Complement activity.** Since it has been demonstrated that hemolytic complement may take part in the process of opsonization, complement activity was compared in the serums of the normal controls and those of children with rickets. The 50 per cent hemolytic units of complement are seen to be similar in these two groups (Fig. 3).

**Test for serum phagocytosis inhibitor.** In view of marked depression of phagocytosis in rickets, an inhibitor was considered and tested

in 5 cases with  $\text{Ca} \times \text{P}$  index below 40. If some factor in the test serum had interfered with phagocytosis serum added to normal serum should have depressed phagocytosis. As seen in Table 2, this was not the case.

**Immunoglobulin levels** The average values of IgG in three age groups (2-3 4-5 6-8 months) were 520 mg/430 mg and 510 mg/100 ml serum by rickets and 480 mg/410 mg and 540 mg/100 ml serum by controls; the levels of IgM 42 mg/49 mg and 51 mg/100 ml serum by rickets and 40 mg/53 mg and 50 mg/100 ml serum by controls. No significant differences were noted between these two groups.

### DISCUSSION

Rickets occurs during the suckling period at a time when immunological reactions against many antigens are not yet developed (15). When looking for the origin of decreased serum opsonizing activity for *Escherichia coli* and interpreting the results in relation to the age, it is necessary to realize that normally the development of an intestinal flora is essential to provoke antibody formation against this bacteria (17). The infant begins to produce his own antibodies against *E. coli* during the 2nd to 4th month of life (10). Because antibodies against gram-negative bacteria which take part in serum opsonizing activity are predominantly gamma M globulins (14), the role

Table 2. Phagocytosis of *E. coli* in various combinations of normal serum and serum from patients with rickets

Serum			
Rickets	Normal	Buffer	of phag
—	0.1 ml	0.4 ml	51
0.4 ml	0.1 ml	—	51
0.4 ml	—	0.1 ml	51
0.1 ml	—	0.4 ml	38

of placental transport is minimal. These antibodies are supplied in various mammals such as the cow and pig via colostrum, but there is no appreciable absorption by the suckling human baby (9). The O antigen of *E. coli* is necessary for the production of opsonins, the titres of which rise during the first year of life (11). For the opsonisation of a smooth strain of *E. coli* which is used in this study, the presence of a specific antibody is important (12). In spite of the existence of many intestinal *E. coli* strains during the suckling period, which differ in every individual (20), the dependence of serum opsonizing activity for *E. coli* B 2340 was observed with respect to the heat-stable component.

Due to the clinical symptoms of rickets varying in intensity, the varying ages of the tested patients and since there are no signs of rickets among the healthy children, the phagocytosis values must be related to some constant. Therefore, in agreement with Fanconi (4), the  $\text{Ca} \times \text{P}$  index was applied. As our results indicate, rickets is accompanied by decreased phagocytosis, which is dependent on age and  $\text{Ca} \times \text{P}$  index. This is due to a lower level of the heat-stable component of opsonin—i.e. an antibody. The fact that the heat-labile component is the same as in healthy children is verified by parallel determination of complement, which is supposedly the main component of the heat-labile opsonin (16). The reason for decreased opsonisation is not the decrease of total immunoglobulin levels; no differences were found in total serum IgG and IgM against controls.

The levels of agglutinins for *E. coli* in the first months of life are either very low or zero.

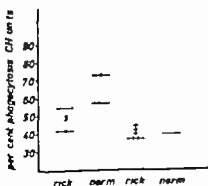


Fig. 3. Phagocytosis of *E. coli* in various sera and complement levels. Tests in heated sera are indicated by solid dots and those in unheated sera by open circles. Complement levels are indicated by crosses.

## REFERENCES

- (19) Based upon "in vivo" clearance studies with *E. coli* it was shown however that the opsonizing ability of the immune serum was a more sensitive measure of antibody activity than agglutination (2). The cause of the decreased serum opsonizing activity in rickets may be a certain impairment of the specific component of immunity response of the organism. Because of the factors which influence quantity and composition of the intestinal bacteria of infants (3) there is no reason to expect differences in antigenical stimulation between normal children and those with rickets. It has been proven that the concentration of calcium influences phagocytosis in vitro (5). However this fact can not play a role in the case of rickets because of very small differences of blood calcium levels in comparison with normal children. Also according to our results the bactericidal capacity in rickets does not change. Leukocytes are functionally fully competent, and additionally there was no evidence for the existence of a serum inhibitor of phagocytosis. Thus the alterations of phagocytosis in rickets seem to be predominantly the consequence of an impaired antibody production.
- ### SUMMARY
- Children with rickets have been shown to have the normal bactericidal capacity of leukocytes for *Escherichia coli* but impaired phagocytosis of this bacteria. It has been proven that the heat stable component of opsonins is responsible for this fact. Dependence of serum opsonizing activity on age and Ca x P index in rickets have been observed. The findings provide a possible explanation for clinical observations on diminished resistance to infection in rickets.
- ### ACKNOWLEDGEMENTS
- We are indebted to Prof. E. Wecker for helpful criticism and supply of bacterial strains. Prof. E. Goltner for advice and help in statistical matters and Dr H. Neumayer for referring 2 patients. We are also indebted to Prof. H. Schelp for his help in English correction.
1. Becker W., Rapp W., Schwack H. G. & Sponko L. Methoden zur quantitativen Bestimmung von Plasmaproteinen durch Immunpräzipitation. *Z. Klin. Chem.* 6: 113 1968.
  2. Benacerraf B., Sebastian M. M. & Schloeman S. A quantitative study of the kinetics of blood clearance of P<sup>32</sup> labelled *E. coli* and *Staphylococcus* by the reticuloendothelial system. *J. Exp. Med.* 110: 27 1959.
  3. Braum O. H. Ernährung und Darmflora von Säuglingstier. *Medizin und Ernährung* 3: 83 1962.
  4. Fomon O. & Wallgren A. *Lehrbuch der Pädiatrie*. Schwabe & Co. Verlag, Basel und Stuttgart 1967 8th edition p. 171.
  5. Henon M. & Delamary A. Action exercée in vitro par les ions Ca<sup>++</sup> et Mg<sup>++</sup> sur la sensibilité chimiotactique et le pouvoir phagocytaire de leucocytes polymorphes. *Ann. Inst. Pasteur* 111: 85 1964.
  6. Hovels O., Bock H., Rasche F. & Sommerhage O. Über das Häufigkeit weiterer rachitischer Zeichen bei Knochentuberkulose. *Z. Kinderheilk.* 69: 361 1951.
  7. Labat E. & Meyer M. M. *Experimental immunology*. Thomas, Springfield, Illinois 1961 p. 149.
  8. Khazanova T. M. The immunoreactive condition of children suffering from rickets. *Pediatr. Akad. Ginek. (Mosk.)* 6: 26 1967.
  9. Kamm C. M. Antibody distribution against non enteropathogenic *E. coli*. *Arch. Intern. Med.* 110: 676 1962.
  10. Loshakov K., Iosya V. & Lenc A. Influence of the intestinal flora on the development of immune reactions in infants. *J. Biol.* 93: 797 1967.
  11. Michael J. G. & Rosen F. S. Association of natural antibodies to gram negative bacteria with the  $\gamma$  macroglobulins. *J. Exp. Med.* 118: 619 1963.
  12. Miller I., Tashkova, H., Mandel L. & Travnick J. The phagocytic activity of reticuloendothelial system of newborn precolostrated pigs to smooth and rough *E. coli*. *Folia Microbiol.* 13: 472 1968.
  13. Miller M. E. & Buckler J. M. Intracellular survival and growth of *Serratia marcescens* following phagocytosis by human leukocytes. *J. Infect. Dis.* 118: 149 1968.
  14. Robbins J. B., Kenny J. & Soter E. The isolation and biological activities of rabbit gamma M and gamma G anti-*Salmonella typhimurium* antibodies. *J. Exp. Med.* 122: 385 1965.
  15. Smith R. T. & Eitzman D. V. Characterization of the response of the human infant and adult to immunization with *Salmonella vaccines*. *Pediatr.* 33: 163 1964.
  16. Stern I. The opsonic activity of complement in sera without antibody. *Folia Microbiol.* 8: 240 1963.

in 5 cases with  $\text{Ca} \times \text{P}$  index below 40. If some factor in the test serum had interfered with phagocytosis, serum added to normal serum should have depressed phagocytosis. As seen in Table 2, this was not the case.

**Immunoglobulin levels** The average values of IgG in three age groups (2-3 4-5 6-8 months) were 520 mg/430 mg and 510 mg/100 ml serum by rickets and 480 mg/410 mg and 540 mg/100 ml serum by controls; the levels of IgM 42 mg/49 mg and 51 mg/100 ml serum by rickets and 40 mg/53 mg and 50 mg/100 ml serum by controls. No significant differences were noted between these two groups.

## DISCUSSION

Rickets occurs during the suckling period at a time when immunological reactions against many antigens are not yet developed (15). When looking for the origin of decreased serum opsonizing activity for *Escherichia coli* and interpreting the results in relation to the age, it is necessary to realize that normally the development of an intestinal flora is essential to provoke antibody formation against this bacteria (17). The infant begins to produce his own antibodies against *E. coli* during the 2nd to 4th month of life (10). Because antibodies against gram-negative bacteria which take part in serum opsonizing activity are predominantly gamma M globulins (14), the role

Table 2. Phagocytosis of *E. coli* in various combinations of normal serum and serum from patients with rickets

Serum			
Rickets	Normal	Buffer	of phag.
—	0.1 ml	0.4 ml	54
0.4 ml	0.1 ml	—	61
0.4 ml	—	0.1 ml	51
0.1 ml	—	0.4 ml	38

of diaplascentar transport is minimal. These antibodies are supplied in various mammals such as the cow and pig via colostrum, but there is no appreciable absorption by the suckling human baby (9). The O antigen of *E. coli* is necessary for the production of opsonins, the titres of which rise during the first year of life (11). For the opsonisation of a smooth strain of *E. coli* which is used in this study, the presence of a specific antibody is important (12). In spite of the existence of many intestinal *E. coli* strains during suckling period, which differ in every individual (20), the dependence of serum opsonizing activity for *E. coli* B 2340 was observed with respect to the heat-stable component.

Due to the clinical symptoms of rickets varying in intensity, the varying ages of the tested patients and since there are no signs of rickets among the healthy children, the phagocytosis values must be related to some constant. Therefore, in agreement with Fanconi (4), the  $\text{Ca} \times \text{P}$  index was applied. As our results indicate, rickets is accompanied by decreased phagocytosis, which is dependent on age and  $\text{Ca} \times \text{P}$  index. This is due to a lower level of the heat-stable component of opsonin—an antibody. The fact that the heat-labile component is the same as in healthy children is verified by parallel determination of complement, which is supposedly the main component of the heat-labile opsonin (16). The reason for decreased opsonisation is not the decrease of total immunoglobulin levels; no differences were found in total serum IgG and IgM against controls.

The levels of agglutinins for *E. coli* in the first months of life are either very low or zero.

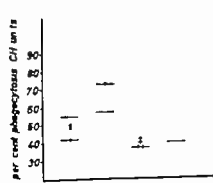


Fig. 3. Phagocytosis of *E. coli* in various sera and complement levels. Tests in heated sera are indicated by solid dots and those in unheated sera by open circles. Complement levels are indicated by crosses.

(19) Based upon "in vivo" clearance studies with *E. coli* it was shown however that the opsonizing ability of the immune serum was a more sensitive measure of antibody activity than agglutination (2). The cause of the decreased serum opsonizing activity in rickets may be a certain impairment of the specific component of immunity response of the organism. Because of the factors which influence quantity and composition of the intestinal bacteria of infants (3) there is no reason to expect differences in antigenical stimulation between normal children and those with rickets. It has been proven that the concentration of calcium influences phagocytosis *in vitro* (5). However this fact can not play a role in the case of rickets because of very small differences of blood calcium levels in comparison with normal children. Also according to our results the bactericidal capacity in rickets does not change. Leukocytes are functionally fully competent and additionally there was no evidence for the existence of a serum inhibitor of phagocytosis. Thus the alterations of phagocytosis in rickets seem to be predominantly the consequence of an impaired antibody production.

### SUMMARY

Children with rickets have been shown to have the normal bactericidal capacity of leukocytes for *Escherichia coli* but impaired phagocytosis of this bacteria. It has been proven that the least stable component of opsonin is responsible for this fact. Dependence of serum opsonizing activity on age and Ca  $\times$  P index in rickets have been observed. The findings provide a possible explanation for clinical observations on diminished resistance to infection in rickets.

### ACKNOWLEDGEMENTS

We are indebted to Prof. E. Wecker for helpful criticism and supply of bacterial strains, Prof. E. Goltner for advice and help in statistical matters and Dr. H. Newmayer for referring 2 patients. We are also indebted to Prof. H. Schelp for his help in English correction.

### REFERENCES

1. Becker W, Rapp W, Schwack H G & Skrifko A. Methoden zur quantitativen Bestimmung von Plasmaproteinen durch Immunprecipitation. *Z. Klin. Chem.* 6: 113 1968.
2. Benacerraf B, Sebestyen, M M & Schloesman S. A quantitative study of the kinetics of blood clearance of  $P^{32}$  labelled *E. coli* and *Staphylococcus* by the reticuloendothelial system. *J. Exp. Med.* 110: 27 1959.
3. Braun O H. Ernährung und Darmflora im Säuglingsalter. *Medizin und Ernährung* 3: 83 1962.
4. Fanconi G & Wallgren A. *Lehrbuch der Pädiatrie*. Schwabe & Co. Verlag, Basel and Stuttgart 1967. 8th edition, p. 171.
5. Henson M & Delaunay A. Action exercée *in vitro* par les ions  $Ca^{++}$  et  $Mg^{++}$  sur la sensibilité chimotactique et le pouvoir phagocytaire des leucocytes polynucléaires. *Ann. Inst. Pasteur* 111: 85 1966.
6. Hovels O, Bock H, Rasche F & Sommerhage O. Über die Häufigkeit weiterer rachitischer Zeichen bei Kränkeltätern. *Z. Kinderheilk.* 69: 361 1951.
7. Kabat E & Mayer M M. *Experimental immunochromatography*. Thomas, Springfield, Illinois 1961, p. 149.
8. Khasanova T M. The immunoreactive condition of children suffering from rickets. *Pediatr. Akad. Ginek. (Mosc.)* 6: 26 1967.
9. Kamm C M. Antibody distribution against non enteropathic *E. coli*. *Arch. Intern. Med.* 110: 676 1962.
10. Lednitskaya R., Jouss V & Lanc A. Influence of the intestinal flora on the development of immune reactions in infants. *J. Bact.* 93: 797 1967.
11. Michael I G & Rosen F S. Association of natural antibodies to gram negative bacteria with the  $\gamma$  macroglobulins. *J. Exp. Med.* 118: 619 1963.
12. Mider I, Tleskalova, H, Mandel L & Travnick J. The phagocytic activity of reticuloendothelial system of newborn precolostrual pigs to smooth and rough *E. coli*. *Folia Microbiol.* 13: 472 1968.
13. Müller M E & Bockler J M. Intracellular survival and growth of *Salmonella marcescens* following phagocytosis by human leukocytes. *J. Infect. Dis.* 118: 149 1968.
14. Robbins J B, Kenny A & Seiler E. The isolation and biological activities of rabbit gamma M and gamma G anti-*Salmonella typhimurium* antibodies. *J. Exp. Med.* 122: 385 1965.
15. Smith R T & Ertzman D V. Characterization of the response of the human infant and adult to immunization with *Salmonella vaccinae*. *Pediatr.* 1963: 33: 163 1964.
16. Szard J. The opsonic activity of complement in sera without antibody. *Folia Microbiol.* 8: 40 1963.



- 17 Stetson C A *Endotoxins and bacterial allergy. Cellular and humoral aspects of the hypersensitive state* Hoeber medical division Harper & Row Publishers New York 1959
- 18 Stroder J Lange F C Emmerling P & Finger H Über Antikörperbildung bei Rachitis *Z Kinderheilk* 107 165 1969
- 19 Stulberg C B Zuelzer W W & Page R H *Escherichia coli* 0127 B8 a serotype causing infantile diarrhea *J Immunol* 76 281 1955
- 20 Vosti K L Monto A S & Rantz L A Sero logic classification of *E coli* The importance of sample size in clinical studies *Clin Res Proc* 10 113 1962
- 21 Winkelstein J A & Drachman R H Deficiency of pneumococcal serum opsonizing activity in sickle-cell disease *New Eng J Med* 279 457 1968

Submitted Aug 27 1969

Accepted Nov 17 1969

Dept of Paediatrics  
Luitpold Krankenhaus  
Josef Schneider Straße 33  
87 Würzburg  
West Germany

*Key words* Rickets phagocytosis opsonisation

## FINE NEEDLE ASPIRATION BIOPSY OF HUMAN LIVER FOR ENZYMIC DIAGNOSIS OF GLYCOGEN STORAGE DISEASE AND GARGOYLISM

A LUNDQVIST and P A ÖCKERMAN

From the Departments of Internal Medicine and Clinical Chemistry, University Hospital, Lund, Sweden

As has already been discussed in an earlier paper (10) there is a great need for safe and accurate methods for the diagnosis of glycogen storage disease. The advent of diagnostic assays on blood cells has not made unnecessary analyses on liver biopsy specimens in many cases. In order to be able to use a biopsy technique involving less risk for the patients than those hitherto used the fine needle aspiration biopsy technique as described by Soderstrom (12) was introduced. Methods were worked out (10) allowing the analyses for the diagnosis of glycogen storage disease to be made on the small fragments of liver tissue obtained.

In earlier papers (5, 6) normal values were given of several enzymes of glycogen metabolism and glucose production as well as enzymes localized intracellularly to the lysosomes. This communication presents the application of the technique in the diagnosis of glycogen storage disease and gargoylism of the Hurler type (mucopolysaccharidosis type I according to McKusick (7)). In this latter disease a deficiency of the activity of  $\beta$  galactosidase has recently been demonstrated (11).

### MATERIAL AND METHODS

#### *Biopsy technique and enzyme assay*

As in ref. 5 and 6 the patient was fasted over night. In the morning a fine needle aspiration biopsy was

performed once or twice in local anesthesia. The procedure for biopsy and immediate preparation of the specimen obtained was as described in an earlier communication (5).

One homogenate in sucrose-EDTA was used for analyses of glucose 6-phosphatase, phosphorylase, arylsulphatase,  $\alpha$ -glucosidase and protein as described earlier (10) and a second homogenate in water for assay of glycogen  $\alpha$ -glucosidase (10),  $\beta$  galactosidase,  $\beta$  glucuronidase,  $N$ -acetyl  $\beta$  glucosaminidase (11) and protein (4). The fact that the fragments were not weighed minimized the risk for inactivation of labile enzymes. Accurate washing would also have been impossible since washing from blood was necessary. So some EDTA was necessary to stabilize glucose 6-phosphatase (5) water to activate all lysosomal enzymes (6).

#### *Patients*

All patients with glycogen storage disease had symptoms and signs in accordance with a diagnosis of hepatomegalic glycogenosis. Glycogen and enzyme assays were performed with earlier described methods (2, 3, 9) on liver (cases 1, 3 and 4) and muscle biopsies (cases 1 and 2) and allowed a definite diagnosis to be made of glycogenosis type I in case 4 (a sister has been reported in case 7 in ref. 9), type III in case 2 and type VI in cases 1 and 3.

The patients with gargoylism all had symptoms and signs in accordance with a diagnosis of Hurler's syndrome and excreted large amounts of glycosaminoglycans (13) mainly dermatan sulfate and to a less extent heparan sulfate (8) in their urine.

### RESULTS

#### *Glycogen storage disease*

Fragments of liver tissue from case 1 obtained by fine needle aspiration biopsy were used for

- 17 Stetson C A *Endotoxins and bacterial allergy Cellular and humoral aspects of the hypersensitive state* Hoeber medical division Harper & Row Publishers New York 1959
- 18 Stroder J Lange F C Emmerling P & Finger H Über Antikörperbildung bei Rachitis *Z Kinderheilk* 107 165 1969
- 19 Stalberg C S Zuelzer W W & Page R H *Escherichia coli* 0127 B8 a serotype causing infantile diarrhea *J Immunol* 76 281 1955
- 20 Vouts K L Monto A S & Rantz L A Sero logic classification of *E coli* The importance of sample size in clinical studies *Clin Res Proc* 10 113 1962
- 21 Winkelstein J A & Drachman R H Deficiency of pneumococcal serum opsonizing activity in sickle cell disease *New Eng J Med* 279 457 1968

Submitted Aug 27 1969

Accepted Nov 17 1969

Dept of Paediatrics  
Luitpold Krankenhaus  
Josef Schneider Straße 2  
87 Würzburg  
West Germany

Key words Rickets phagocytosis opsonisation

## DISCUSSION

The results given for comparison represent to our knowledge the only values published for strictly normal healthy individuals. However all controls were adults and may therefore not represent an optimal reference. In spite of its relative freedom from risks to the patients fine needle aspiration biopsies were not considered feasible in normal children. Also it is to be expected that unspecific changes in the form of increased or decreased values for some enzyme activities will be obtained as a result of the disease. Consequently only very clear differences can be easily interpreted. We consider that such clear differences existed in the seven patients described in this communication.

Since the first enzyme defect in glycogen storage disease was described in 1952 (1) a development of the methods of enzyme assays has occurred. The amount of tissue needed has diminished. Thus it was considered a great step forward when Hers in 1959 (2) published methods allowing the assay of glycogen glucose 6-phosphatase, amylo-1,6-glucosidase and phosphorylase to be made on only about 10–20 mg of tissue. To be able to analyse a glucosidase a further 5–10 mg of liver was needed (3). The present methods allowing analyses on 0.5–2 mg thus represents a sensitivity at least 10 times higher.

The main advantage of the suggested procedure is that it is less risky to the patient than other liver biopsy procedures. The following drawbacks can be mentioned: 1) The assays are slightly more time-consuming than Hers methods (2, 3). 2) It has not been proved that samples can be sent from one hospital to another. 3) No liver tissue can be saved frozen for future use since all the material obtained is used for the analyses.

Nevertheless the suggested procedure would represent a recommendable technique in cases of metabolic diseases in which analyses of more easily obtainable tissue such as blood cells are not sufficient or when general anaesthesia or larger diameter needle biopsy is considered dangerous.

## SUMMARY

Using a needle outer diameter 0.7 mm liver biopsies were performed in local anaesthesia. The small fragments of tissues obtained 0.5–2 mg were used for assay of enzymes and glycogen in one case each of glycogenosis type I and II and two of type VI and three cases of Hurler's syndrome. The enzyme defects known to exist in liver in these diseases could be demonstrated while no defects were found of related enzymes. It is suggested that fine needle aspiration biopsy of the liver should be used for diagnostic purpose with assay of relevant enzymes in glycogen storage disease and gargoylism and maybe also other metabolic diseases. This method involves less risk for the patient than conventional liver biopsy procedures and gives information that in many cases cannot be obtained by analyses on other tissues.

## ACKNOWLEDGEMENT

The skilful technical assistance of Mrs C Lindfors, Miss S Olsson and Miss M Spolander is gratefully acknowledged. This investigation was supported by the Swedish Medical Research Council (Grants 367 19X 222-01 and B68 13X 2307-01). The University of Lund and Fortsmåpharmaceutiska Riksförbundet.

## REFERENCES

- 1 Cori G T. Glycogen Structure and enzyme deficiencies in glycogen storage disease. *Harvey Lectures* 46 145 1952/53.
- 2 Hers H G. Fraides enzymatiques sur fragments hépatiques. Application à la classification des glycogénoses. *Rev Internat Hepatol* 9 35 1959.
- 3 — a glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). *Biochem J* 86 11 1963.
- 4 Lowry O H, Rosebrough N J, Farr A L & Randall R J. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193 265 1951.
- 5 Lundquist A, Öckerman P A & Schersten B. Fine needle aspiration biopsy of the liver in healthy adults. Activity of enzymes of glycogen utilization and glucose production. *Enzym Biol Clin* 10 8 1969.
- 6 Lundquist A & Öckerman P A. Fine needle aspiration biopsy of the liver in healthy adults. Activity of lysosomal acid hydrolases. *Enzym Biol Clin* 10 300 1969.

Table 1 Enzyme activities and glycogen concentration in human liver

Enzyme activities expressed as  $\mu$ moles substrate split/g protein/min except for amylase 1-6-glucosidase given as  $\mu$ moles product formed. Glycogen concentration given as g/g protein

		Glucose 6-phosphatase	Phosphorylase	Amylo 1-6-glucosidase	$\alpha$ -glucosidase	Glycogen
Controls <sup>a</sup> n = 14-16	Mean Range	41.6 (23.5-73.8)	128.7 (72.8-230.3)	4.20 (2.08-7.49)	14.2 (2.49-34.6)	0.206 <sup>b</sup> (0.096-0.3)
<i>Glycogen storage disease</i>						
Case 1 male 5		15.9	36.6	1.60	1.30	0.609
Case 2 male 1		8.4	280.0	0	10.4	2.34
Case 3 female 1		36.7	36.2	2.97	—	2.12
Case 4 female 1		0.0	160.0	2.92	4.71	1.28

<sup>a</sup> Healthy adults (5)

<sup>b</sup> Biopsy taken at operation (10)

analyses of enzyme patterns and glycogen as shown in Table 1. It is evident that the glycogen concentration was increased. The activity of glucose 6-phosphatase, amylase 1-6-glucosidase and  $\alpha$ -glucosidase was only slightly decreased, a very definite activity still remaining. This excludes a diagnosis of glycogenosis type I, II and III. Phosphorylase activity was decreased to about 20% of the mean activity in the controls. This is in good accordance with a diagnosis of glycogenosis type VI, where usually one finds increased glycogen and a decrease of the activity of phosphorylase to 1/2 to 1/4 of the normal. It is concluded that a correct diagnosis could be made on the results of the analyses on the fine needle biopsy specimen.

In case 2 a very high level of glycogen was found. Glucose 6-phosphatase activity was

slightly decreased, a very definite activity remaining.  $\alpha$ -glucosidase and phosphorylase activities were high. No activity of amylase 1-6-glucosidase was found. The results are in accordance with a diagnosis of glycogen storage disease type III. In case 3 a similar reason as in case 1 can be applied and a diagnosis can be made of glycogen storage disease type VI.

The findings in case 4 very clearly are in accordance with a diagnosis of glycogen storage disease type I.

#### Gargoylism

Table 2 shows the result in the patients with gargoylism. Clearly a deficiency of  $\beta$ -galactosidase could be diagnosed from these results, while the activities of  $\beta$ -glucuronidase and N-acetyl  $\beta$ -glucosaminidase were not decreased.

Table 2 Activity of acid hydrolases in fine needle biopsies of human liver

All values expressed as  $\mu$ moles substrate split/g protein/min

		$\beta$ -galactosidase	$\beta$ -glucuronidase	N-acetyl $\beta$ -glucosaminidase
Controls <sup>a</sup> (n = 24)	Mean (Range)	4.79 (1.69-11.7)	4.32 (2.15-9.16)	13.3 (7.59-29.9)
<i>Gargoylism</i>				
Case 1 female 8		0.27	1.98	13.5
Case 2 female 5		0.48	9.25	75.6
Case 3 female 8		0.88	14.1	90.2

<sup>a</sup> Healthy adults (6)

## CYTOMEGALOVIRUS INFECTIONS IN DIFFERENT GROUPS OF PAEDIATRIC PATIENTS

CUN CARLSTRÖM and BIRGITTA JÄLLING

*From the Section of Virology Department of Clinical Bacteriology and the Department  
of Paediatrics Karolinska Spitalhuset Stockholm Sweden*

According to previously presented findings cytomegalovirus (CMV) infections frequently occur in the Swedish population (2). The high proportion of individuals found to carry antibodies against CMV corresponds well with findings from other geographical areas (10, 16, 19). The introduction of viral diagnostic procedures for CMV infections has been followed by a gradual delineation of the clinical picture of both congenital (5, 9, 12, 14, 18, 21) and acquired (1, 3, 4, 5, 7, 8, 10, 11, 12, 13, 17, 20) CMV infections. However many problems await further elucidation. The role of CMV as a pathogen in different clinical syndromes has not yet been completely explored nor has the incidence of inapparent CMV infections and minor clinical illnesses with subtle or unspecific manifestations been thoroughly investigated. Because of observations of the persistence of infection and the reactivation of dormant virus it is difficult to assess both in acute and chronic cases whether the disease is caused by a primary infection or a reactivation. Furthermore the mode of transmission of infection is yet not fully understood.

The present investigation was undertaken in an attempt to elucidate some of these problems. Sero-epidemiological and virus isolation studies were carried out at Karolinska sjukhuset from 1963 through 1967 in different groups of children.

## MATERIAL AND METHODS

*Clinical material.* Seven groups of subjects were studied.

*Group 1.* Newborn infants with neonatal jaundice but without blood group incompatibility and without other signs of illness.

*Group 2.* Infants up to six months of age with suspected infectious disease and CMV infection questioned.

*Group 3.* Children seven months to fifteen years of age admitted with various acute infectious diseases.

*Group 4.* Children seven months to five years of age admitted to the Ear, Nose and Throat Department with acute subglottic laryngitis.

*Group 5.* Seven months to fifteen years old children with congenital or acquired disease of the central nervous system admitted for neurology study.

*Group 6.* Seven months to fifteen years old children, admitted because of congenital heart disease.

*Group 7.* Adults, nursing staff or family members of diagnosed or suspected cases of CMV infection.

*Virological techniques.* Used in the diagnostic tests for CMV were those previously described (2). CMV was looked for in urine and/or throat specimens and serological diagnosis was made by complement fixation tests (CFT) with paired acute and convalescent sera or in newborn infants with repeated sera collected at two-month intervals. Some patients were studied by both virus isolation and serological methods; sometimes only one of the diagnostic procedures was applied.

*Isolation and identification of CMV.* Throat swabs collected in 15 ml of virus transport medium (gelatin lactalbumen hydrolysate yeast extract medium with penicillin, streptomycin and nystatin) and equal parts of virus and transport medium respectively were inoculated within a couple of hours after collection into diploid human embryonic fibroblasts. Each tissue culture tube was inoculated with 0.5 ml and

- 7 McKusick V A *Heritable disorders of connective tissue* C V Mosby Co Saint Louis 1966 3rd edition
- 8 Manley G & Hawksworth J Diagnosis of Hurler's syndrome in the hospital laboratory and the determination of its genetic type *Arch Dis Child* 41 91 1966
- 9 Öckerman P A Glycogen storage disease in Sweden *Acta Paediat Scand Suppl* 160 1965
- 10 — A technique for the enzymatic diagnosis of glycogen storage disease on very small tissue specimens *Acta Paediat Scand* 57 105 1968
- 11 — Lysosomal acid hydrolases in the liver in gargoyism Deficiency of 4-methylumbelliferyl  $\beta$ -galactosidase *Scand J Clin Lab Invest* 22 142 1968
- 12 Söderström N *Fine needle aspiration biopsy* Grune & Stratton New York 1966
- 13 Teller W M Burke H C Rosevear J W & McKenzie B F Urinary excretion of acid mucopolysaccharides in normal children and patients with gargoyism *J Lab Clin Med* 59 88 1962

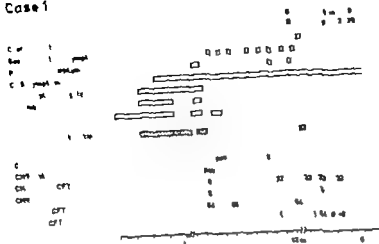
Submitted Sept 25 1969

Accepted Nov 11 1969

(P A Ö) Dept of Clinical Chemistry  
Lasarettet  
220 05 Lund  
Sweden

**Key words** Fine needle aspiration biopsy glycogen storage disease gargoyism liver enzymatic diagnosis

## Case 1



## Case 2

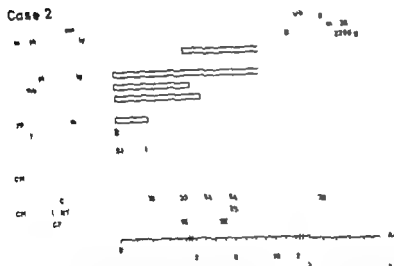


Chart 1. Relevant data in two children with congenital CMV infection

performed as proper serum samples were not obtained. In two of the seronegative cases the CMV suspected agent was found to be herpes simplex. On four occasions adenovirus was isolated; this virus however causing a different cytopathogenic pattern in the fibroblasts.

#### Distribution of CMV antibodies

Complement fixing titres of single sera from patients of all ages were used to study the distribution of CMV antibodies in different groups of patients. Previously published data on the antibody pattern of 242 patients of different ages selected at random in Karolinska Spitalhuset (1) were included in this material.

The results of the in all 811 human sera studied with CFT are recorded in Fig. 1.

The intention was to compare the CMV antibody pattern in children with congenital heart diseases and central nervous system diseases with that of the rest of the patients. Children with heart diseases were found to have an equivalent pattern to that of the main group and were therefore included while a higher percentage of seropositive individuals was found at all ages among patients with neurological diseases (Fig. 1). This difference however was not significant and from this material no conclusions can be drawn as to the role of CMV in the aetiology of neurological diseases.



Table 1 Incidence of CMV infection in different groups of patients

Group	Number of patients tested					Total patients tested	CMV positive patients
	By virus isolation from			By serology			
	Throat	Urine	Throat + urine	CFT + isol	CFT only		
I (newborn infants jaundiced)		105	13	13*	10	128	0
II (0-6 mo infections suspected)		29	19	21	14	62	2
3 (7 mo-15 yr acute infections)		24	60	18	12	96	2
4 (7 mo-5 yr pseudocroup)	194					194	0
5 (7 mo-15 yr cerebral damage)	4		30			34	0
6 (7 mo-15 yr cong heart disease)	11	2	44			57	0
7 (well adult contacts)	10	12	14	6	6	42	0
Total						613	4

\* For obvious reasons the figures in this column are not included in the totals

the maintenance medium was changed at intervals of one week. Inoculated cultures were examined once a week for two months and positive cultures were passaged by transference of infected cells to new tissue cultures. After four passages CMV could usually be transferred by the fluid medium. Isolated strains which caused CMV typical cytopathogenic effects in fibroblasts were tried in HeLa and GMK cells and when negative were checked by neutralization tests (NT) against paired acute and convalescent sera from patients with previously diagnosed CMV infection.

**Serological tests.** In CFT and NT the Ad 169 strain of CMV was used. Paired acute and convalescent sera were tested simultaneously as were sera from a mother and her newborn infant as well as repeated sera from the infants. CFT was used for primary screening. A fourfold rise in titre was taken as a significant change. All sera were tested at two fold dilution steps 1/2 being the lowest dilution. When CMV infection was diagnosed by CFT NT was done.

## RESULTS

### Prevalence of CMV infection

Virological investigation for CMV infection was performed in 613 hospital admissions, four positive cases being found. Two of these were diagnosed by virus isolation and serology and two by serology only. The incidence of CMV infection in the different groups of patients is given in Table 1.

Two of the positive cases were infants rep-

resenting congenital CMV infection and two belonged to Group 3 i.e. older children with symptoms of acute infection.

**Congenital infection.** As previous reports on congenital CMV infections are now numerous relevant data on our two cases are summarized in Chart 1.

**Acquired infection.** Two children 4 and 9 years of age had CMV infection running the course of an acute acquired infectious disease. These patients also had severe chronic diseases and were both on treatment with cortisone. Relevant data on these cases are summarized in Chart 2. A detailed case description will be published elsewhere.

### Virological diagnosis of CMV infection

In virus isolation trials cytopathogenic effects with focal lesions suggestive of CMV were found in 14 patients. Three of them (and one with negative isolation trial) were seropositive for CMV (rise in titre from acute to convalescent phase) and in two of the three cases CMV could be subcultured. In 12 patients subpassage of CMV suspected agent could not be accomplished. Serological investigations for CMV were positive in two and negative in 8 of these 12 cases. In two cases serology was not

Table 2 CMV antibodies in mothers and their infants

	Mothers at delivery	Infants		
		At birth	After 1-3 months	After 4-6 months
Number of seropositive subjects (CFT 4-64)	23	19/19	4/14	0/6
Number of seronegative subjects (CFT <2)	11	11/11	5/5	—

Denominator indicates total number tested in each group

#### Maternal CMV antibody titre

Results of studies on transplacental passage of CMV antibodies and the persistence of maternal titres are given in Table 2

In many cases transplacental antibodies disappeared within a couple of months and were not found to persist after 4 months

### DISCUSSION

The epidemiology of CMV infection has been studied from the point of view of virus excretors in many differently selected hospital materials. Row *et al.* (15) found healthy newborns and adults to be negative but demonstrated relatively high CMV isolation rates (28%) among children 6 months-4 years of age institutionalized for socio-economic reasons. These results were in principle confirmed by Hershaw *et al.* (7) who furthermore demonstrated a high incidence of excretors among children who were contacts to patients excreting CMV. Adult contacts were negative. Stern (18) in a study of unselected hospital admissions in London found that 10% of children between 2 months and 5 years old were excreting CMV. Only one virus excretor aged 6 years was found among 575 older children and adults.

Many of the virus excreting children reported by other authors were not overtly ill but

on retrospective examination showed different symptoms particularly liver dysfunction respiratory distress and minor illnesses. The majority recovered completely and Stern stresses that, contrary to currently held views this goes also for infections in the early neonatal period. On the other hand Stern reports a patient who developed a severe brain injury after an "unapparent" congenital CMV infection.

In the present study CMV infection was demonstrated in four out of 613 patients and the positive cases were all overtly ill. Two were infants and had all the classical symptoms commonly associated with neonatal cytomegalic inclusion disease. The two older children had acute pneumonitis and suffered from chronic diseases. The four patients were all treated with cortisone. No subclinical cases were found. The incidence of CMV infection in this material is in agreement with the findings of Hershaw *et al.* (7) in healthy and hospitalized children (1 per cent isolation rate) but is low compared to findings in chronically institutionalized patients (7-15) and also compared to the figures of Stern (18) for hospitalized children between two months and five years of age.

The laboratory technique for virus isolation is of interest in this connection. CMV is an unstable agent. Some virologists have tried to increase the sensitivity of the virus isolation technique by using ultracentrifuged urine specimens. This was not done here as it would cause a delay of the inoculation procedure and increase the risk of virus inactivation. A laboratory routine requiring accomplished serial subcultivations of isolated CMV strains may lead to the loss of labile low titre virus in some positive cases. However the sole criterion of typical cytopathogenic effect in isolation trials was not considered quite safe and specific for the diagnosis of CMV nor was positive cytology alone. As a rule typical cytopathogenic agents could be subcultured but unsuccessful subpassages of CMV were found to occur. Occasionally CMV suspected agents were identified as herpes simplex. The two patients with



single sera be considered to be a significant diagnostic criterion

## ACKNOWLEDGEMENT

We wish to express our appreciation to Mrs Gunilla Hedström and Mrs Eva Olsson for competent technical assistance

## REFERENCES

- 1 Andersen J P & Stern H Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis *Brit Med J* 1 612 1966
- 2 Carlström G Virologic studies on cytomegalic inclusion disease *Acta Paediatr Scand* 54 17 1965
- 3 Carlström G Alder J Beltrage S Hedström G Holmberg L Nordberg P & Serner G Acquired cytomegalovirus infection *Brit Med J* 11 51 1968
- 4 Carlström G Beltrage S Ohlsson N M & Svedberg J Cytomegalovirus infection complicating open heart surgery *Scand J Thor Cardiovasc Surg* 2 57 1968
- 5 Hershberg J B Congenital and acquired cytomegalovirus infection *Pediatr Clin N Amer* 13 279 1966
- 6 — Cytomegalovirus complement fixing antibody in macrocephaly *New Eng J Med* 275 476 1966
- 7 Hershberg J B Betts R F Simon G & Boynton R C Aquired cytomegalovirus infection *New Eng J Med* 272 602 1965
- 8 Ironside A G & Tobin J O H Cytomegalovirus infection in the adult *Lancet* 11 613 1967
- 9 Eryu E & Henry G E Cytomegalic inclusion disease of infancy *Pediatrics* 38 957 1966
- 10 Hietala E & Hietanen L Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis *Brit Med J* 11 1099 1965
- 11 Hietala E Hietanen L von Essen R Hallur K Kivimäki A & von Bonsdorff C H Further studies on cytomegalovirus mononucleosis in previously healthy individuals *Acta Med Scand* 182 311 1967
- 12 Knoch U Jung M Jung P & Bingenen Ch Virologische und klinische Untersuchungen bei kongenitalen und postnatalen Cytomegalien *Schweiz Med Wochr* 98 1439 1968
- 13 Lamb S G & Stern H Cytomegalovirus mononucleosis with jaundice as presenting sign *Lancet* 11 1003 1966
- 14 Madeczi D N Jr Observations concerning human cytomegalovirus infection and disease *Bull Hopkins Hosp* 114 181 1964
- 15 Rowe W P, Hartley J W Cramblett H G & Mastropa F M Detection of human salivary gland virus in the mouth and urine of children *Amer J Hyg* 87 57 1958
- 16 Rowe W P Hartley J W Waterman S Turner H C & Haebler R J Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids *Proc Soc Exp Biol* 91 418 1956
- 17 Rausser V & Sarkis P Cytomegalovirus in patients *Lancet* 11 772 1967
- 18 Stern H Isolation of cytomegalovirus and clinical manifestations of infection at different ages *Brit Med J* 1 665 1968
- 19 Stern H & Elek S D The incidence of infection with cytomegalovirus in a normal population *J Hyg* 65 79 1965
- 20 Toftull P S Bailey M E Williams R, Ziegler R & Brown R Cytomegalovirus hepatitis in the adult *Lancet* 1 1351 1967
- 21 Weller T H & Hershberg J B Virologic and clinical observations on cytomegalic inclusion disease *New Eng J Med* 266 1233 1962

Submitted Oct 7 1969

Accepted Nov 12 1969

(G C) Section of virology  
Dept of clinical bacteriology  
Karolinska Sjukhuset  
Stockholm  
Sweden

**Key words** Cytomegalovirus incidence of infection paediatric patients complement fixation test

positive CMV isolations were both on treatment with cortisone a procedure which may improve the conditions for positive isolations. In the present cases it seems possible that cortisone has been life saving in the acute CMV incidences whereas it may have provoked clinical CMV infection in the chronically treated patients.

From the present study previously published experiences (3, 4) and unpublished data—in all comprising 26 positive cases—it may be concluded that in our hands complement fixation tests of paired acute and convalescent sera seem to be the routine diagnostic method of choice for CMV infection. It is preferable also to virus isolation because of the instability of the virus, the particular relations between CMV and its host cell and the chronicity of the virus infection. Combined virus isolation and serology tests are of course preferable.

Serological diagnosis alone may be accomplished also in newborn babies as maternal antibodies are eliminated rather rapidly from the infant's blood. As shown in Table II most such antibodies were eliminated in 1–3 months and after 4–6 months no persisting titres were found. Therefore a neonatal CMV titre persisting for more than 4 months is to be considered important and should lead to an attempt to isolate virus. Infants as well as older patients have responded to CMV infection with a significant rise in antibody titre and in all diagnosed cases there has been a long lasting persistence of the complement fixing CMV antibodies. Not even in the patient followed for five years was a decrease in titre recorded. This trend has been demonstrated also in our previous publications (4) and differs from the pattern of immune reaction in many other infectious diseases particularly regarding CF antibodies. The chronicity of CMV infection may have a bearing upon this fact.

One of our intentions stimulated by previous reports (6) was to investigate whether the demonstration of CMV antibodies in single sera could be used as a significant diagnostic

criterion in patients less than five years of age. This was not found to be the case since rather many of the children of this age were found to be seropositive. The present material was selected among hospitalized and not chronically institutionalized children. The pattern of their immune response (Fig. 1) indicates a tendency to an early appearance of CMV antibodies 12.5 and 23 per cent of children at months and five years of age respectively were seropositive. Corresponding figures reported by Hanshaw (6) were 2.6 and 6.3 per cent and by Stern & Elek (19) 4 per cent. To investigate more thoroughly whether this reflects a real epidemiological difference in Sweden or whether the present clinical material is selected virus isolation and serological studies for CMV are in progress on children of different ages attending a well baby clinic. One may speculate whether the rather high rate of seropositive children in this study may have a bearing upon the comparatively low rate of virus isolation.

## SUMMARY

The incidence of CMV infection was studied in 613 patients of various ages in Stockholm. Four positive cases were found: two infants with congenital infection and two older children who also suffered from other chronic diseases and were treated with cortisone.

Complement fixation tests of paired acute and convalescent sera have in our hands been the routine diagnostic method of choice for CMV infection. Serological diagnosis alone may be accomplished also in newborn babies as the maternal antibodies were found to be eliminated rather soon from the infant's blood.

The distribution of CMV antibodies at different ages and in different clinical groups of patients was studied by CFT in 811 human sera. There was no significant difference between the various clinical groups. A relatively high percentage of children less than five years of age were found to be seropositive (12.5–23%). Therefore not even in small children can the demonstration of CMV antibodies in

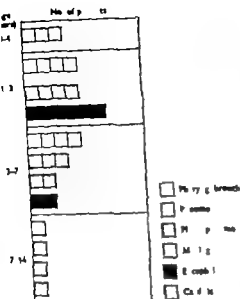


Fig. 1 Clinical diagnosis of adenovirus infections in relation to age

of complement were employed in the test. Serum specimens were also tested in many cases with an antigen of rubinstein A 2 and B streptococcus pneumoniae 1, 2 and 3 respiratory syncytial herpes simplex cytomegalovirus orthonchovirus pox 1, 2 and 3 Coxsackie A 7 Coxsackie B 1, 5 and mycoplasma pneumoniae.

## RESULTS

### Clinical picture

From January to July 1967 32 children with an infection produced by adenovirus type 7 were treated in the Department of Paediatrics University of Oulu. Adenovirus type 7 was isolated from these patients and/or they had a significant (4 fold or higher) rise in titres.

The principal clinical diagnoses were

pharyngobronchitis	10
pneumonia	11
pleuropneumonia	2
meningitis	1
encephalitis	7
carditis	1

Fig. 1 shows the principal clinical diagnosis of these patients in relation to age. Fig. 2 presents the monthly incidence of the adenovirus infections and of encephalomeningitis.



Fig. 2 Monthly incidence of patients with (a) adenovirus infection and (b) adenovirus encephalomeningitis treated in the hospital

Fig. 3 illustrates the typical symptoms and findings in the patients with adenovirus encephalomeningitis. As can be seen the prevalent symptom was fever.

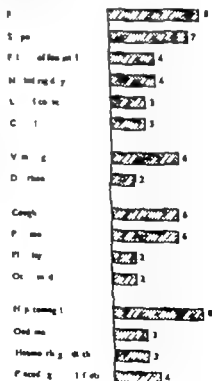


Fig. 3 Clinical picture of adenovirus encephalomeningitis

## ENCEPHALOMENINGITIS IN CHILDREN ASSOCIATED WITH AN ADENOVIRUS TYPE 7 EPIDEMIC

SEPPO SIMILÄ RIITTA JOUPPILA AIMO SALMI and RAILI POHJONEN

*From the Department of Paediatrics University of Oulu and the Department of Virology  
University of Turku Finland*

Adenoviruses usually cause widespread epidemics of acute respiratory disease in military recruits (16) and limited outbreaks among children. Other adenoviral diseases are ceratoconjunctivitis and pneumonia. The latter is a serious and sometimes fatal adenovirus disease in children (18-23). More unusual adenovirus diseases are myocarditis, nephritis and meningoencephalitis (2, 3, 4, 15, 25).

Encephalitis associated with adenovirus infection was first described by Lelong *et al* (15) who isolated adenovirus type 7 from the cerebrospinal fluid and brain tissue of fatal encephalitis in a child. Association of adenovirus type 7 with encephalitis has also been reported by Gabrielson *et al* (8) and Jansson *et al* (9). Usually the encephalitis is only one of the many symptoms caused by severe generalized adenovirus infections in children and encephalitis occurs more frequently than meningitis. In addition to type 7 adenovirus types 1, 2, 3, 6 and 12 have been isolated from patients with encephalitis or meningitis (5, 13, 14, 20, 22). Stenger *et al* (24) reported an epidemic caused by an unidentified adenovirus type in a children's home where 15 of the 60 children infected developed encephalomeningitic symptoms.

The present report describes 8 cases of encephalomeningitis or meningitis in infants and children associated with an adenovirus type 7 epidemic in Northern Finland, Pohjanmaa.

## MATERIALS AND METHODS

### *Study population*

The clinical material consisted of all children (79 cases) with an adenovirus type 7 isolation and/or increase in adenovirus CF antibody titre admitted to the Department of Paediatrics, University of Oulu, between January and July 1967. The children came from different families living in the city Oulu and ten surrounding municipalities.

### *Virus isolation*

Throat and anal swab specimens were inoculated into duplicate cultures of HeLa U (continuous line of human amnion cells) and primary monkey kidney cells. These cell cultures were maintained in Earle's MEM with 5% inactivated calf serum, Eagle's MEM with 2.5% chicken serum and medium no. 199 with 0.5% bovine plasma albumin respectively. Cell cultures inoculated with anal specimens were incubated at 37°C and with throat specimens at 34°C for 14 days. The inoculated cultures were examined 3 times a week and the media were changed every 4 to 5 days. Subcultures were made if viral degeneration of the cell was suspected. Hemadsorption tests with human type O erythrocytes were done in monkey kidney cultures. For the identification of virus isolates, cultures with completely degenerated cells were frozen and thawed 5 times and the supernatant was used in the identification tests. Adenovirus isolates were first identified by CF technique using paired human sera with a known rise in CF antibody titre. Further typing of adenovirus isolates was done by a hemagglutination-inhibition test using type-specific rabbit antisera against prototype strains of adenoviruses.

### *Serological tests*

Antibody determinations of paired serum specimens were carried out with complement fixation test according to a slightly modified micromethod of Sever (21). Adenovirus antigen was prepared in HeLa cells with type 5 virus. Four antigen units and 2 full units

Table 2 Cerebrospinal fluid and EEG in patients with convulsions

Case no.	Age	Sex	Clinical symptoms	Cerebrospinal fluid		Sugar (mg/100 ml)	Protein (mg/100 ml)	Taken days after admission to hospital	Findings
				Specimens taken days after admission to hospital	Cells in mm <sup>3</sup>				
1	2 y	M	Fever pneumonia stupor nuchal rigidity	9 0	1 0	86 48	18 12	2 11	Pathological Normal
2	1 y 4 m	M	Fever pleuropericarditis oedema hepatomegaly haemorrhagic diathesis loss of consciousness convulsions death	0 10	0 0	56 52	18 118		
3	8 y	M	Fever pharyngo-bronchitis nuchal rigidity	0 6	560 340	61 53	93 220		
4	1 y 3 m	F	Fever pleuropericarditis oedema hepatomegaly haemorrhagic diathesis loss of consciousness convulsions death	0	0	65	26	2 22	Pathological Pathological
5	1 y 1 m	M	Fever pneumonia os dema hepatomegalia confusion irritability	0	3	56	16	1	Pathological
6	1 y 5 m	F	Fever oedema hepatomegaly ataxia focal signs of focal encephalopathy nuchal rigidity irritability	1	15	41	16		
7	3 y 11 m	F	Fever pleuropericarditis oedema hepatomegaly loss of consciousness confusion death	1	2	59	—		Pathological
8	2 y	M	Fever pneumonia os dema hepatomegalia confusion nuchal rigidity						



Table 1 *Virus isolations and antibody studies in patients with encephalomeningitis*

Case no	Type of specimen	Specimen taken		Adenovirus isolated	Adenovirus CF antibody titre
		Days after onset of illness	Days after admission to hospital		
1	Throat swab	16	7	+	
	Serum	12	3		1/16
	Serum	28	17		1/64
2*	Anal swab	17	3	+	
3	Throat swab	18	9	+	
	Serum	7	0		1/4
	Serum	21	14		1/16
4	Serum	9	1	+	1/8
	Lung (in the autopsy)	9	1		
5	Throat swab	6	1	+	
	Anal swab	6	1		
	Serum	6	1		1/16
	Serum	19	14		1/256
	Serum	27	22		1/256
6	Throat swab	7	1	+	
	Anal swab	7	1		
	Serum	7	1		1/64
	Serum	16	10		1/256
7*	Anal swab	3	1	+	
	Lung (in the autopsy)	9	7		
	Serum	3	1		1/4
8	Throat swab	9	2	+	
	Anal swab	9	2		
	Serum	9	2		1/64
	Serum	17	10		1/128

**Fatal disease**

Convulsions occurred in three patients stupor in seven loss of consciousness in no less than three. All patients had respiratory symptoms. Six had roentgenologically verified pneumonia, and three additional pleurisy. Hepatomegalia and oedema were very often part of the clinical picture. Haemorrhagic diathesis appeared in the most severe cases.

**Virological findings**

Adenovirus type 7 were isolated in all cases (Table 1) in two (cases 1-3) from the nasopharynx only in two (cases 2-7) from the faeces and in three (cases 5-6-8) from both specimens. In one patient it was isolated only from lung tissue taken on autopsy (case 4). No other viruses were isolated from these specimens. A fourfold of greater rise in adenovirus CF antibody titres was seen in six patients. In the

fatal case (case 2) no specimens for adenovirus antibodies were taken but the finding on the autopsy was similar to that of the fatal infections associated with adenovirus reported in literature (3-15-17-20). In one fatal case (case 4) there was time for only one specimen to be taken.

No rise in CF antibody titres of influenza A 2 and B mumps parainfluenza 1-2 and 3 respiratory syncytial herpes simplex cytomegalovirus or ornithosis polio 1-2 and 3 Coxsackie A 7 Coxsackie B 1-5 and mycoplasma pneumoniae was found in the seven patients.

**Clinical laboratory findings**

Cerebrospinal fluid findings are presented in Table 2. Pleocytosis was noted in two patients and protein increased in two ones. The ESR exceeded 20 mm/h in five patients. The

ity cannot be judged. Since however no less than one fourth of the cases requiring admission to hospital had symptoms of the central nervous system it would seem that the rate of meningoencephalitis and meningitis associated with this epidemic was remarkably high.

In the present cases adenovirus type 7 was isolated from the nasopharynx or rectal mucus and in two also from lung tissue. No virus isolations from the cerebrospinal fluid were carried out owing to the distant location of the virus laboratory. Adenovirus type 7 has only once been isolated from cerebrospinal fluid and brain tissue (15).

Virus encephalitis may either be directly caused by the virus or follow after infection (12). Two of the present patients had a recent history of measles or chickenpox. In these cases the encephalitis may have been post infectious and children may have had the respiratory disease caused by the adenovirus. Furthermore epidemic adenovirus type 7 often occurs in closed communities (16). It is therefore theoretically possible that the present patients contracted their adenovirus infection in the hospital while some other agents had caused the initial disease. This assumption is contradicted in many cases by the fact that the adenovirus could be isolated both from the nasopharynx and the rectal mucus as early as 1-2 days after admission. The adenovirus CF antibody titre which on admission were distinctly above the average level also suggest an infection already contracted at home especially since antibodies to other tested viruses had not increased. It seems therefore most probable that the meningoencephalitis at least in the majority of cases was caused by adenovirus type 7.

The prognosis of adenovirus encephalomeningitis is not very well known. The mortality rate is very high in the present series 38 per cent and in Siengera's series 26 per cent (24). Although the clinical picture is severe the patients who survive the acute phase of the disease contract no irreversible lesions (1, 9, 14, 24).

## SUMMARY

Of the 32 children with an adenovirus type 7 isolation and/or a significant rise in adenovirus CF antibody titre treated in the hospital seven had meningoencephalitis and one meningitis. The encephalomeningitis was associated in each case with respiratory illness six had pneumonia and three additional pleurisy. The patients with encephalomeningitis were sent only all three were unconscious three cases were fatal. The prognosis of those surviving was apparently good according to a short observation period.

## REFERENCES

1. Bebe M, Chany C, Habib E C, Mekhou P, Roberson J, Soudais P & Leliong M. Epidémie familiale de maladie à adenovirus type 7 avec manifestations encéphaliques et sanguines. *Arch Franc Pediatr* 21: 87 1964.
2. Cabanel G, Voog R & Philip X. Péricardite aigue avec reaction de deviation du complément positive aux adenovirus. *J Med Lyon* 47: 111 1966.
3. Chany C, Leprieu P, Leliong M, Le Tan Vinh, Sarge P & Vaut J. Severe and fatal pneumonia in infants and young children associated adenovirus infections. *Amer J Hyg* 80: 367 1958.
4. Dahl M, Samula S & Saloni A. Myocarditis associated with adenovirus type 7 infection. To be published.
5. Ehrlich M & Feders R & Kie G. Adenovirus infection (type 12) as a cause of meningoencephalitis. *Z Kinderheilk* 87: 275 1961.
6. Forsvall P, Hakosla H, Sternstrom R, Jansson E & Wager O. An adenovirus epidemic due to types 1 and 2. *Acta Paediatr Scand* 8: 35 1962.
7. Gershon M O, Joseph C & Huang G D. Encephalitis associated with adenovirus type 7 occurring in a family outbreak. *J Pediatr* 68: 142 1966.
8. Gibbs F A & Gibbs E L. *Atlas of electroencephalography* vol 3. Neurological and psychiatric disorders. Addison-Wesley Publ Comp, Long 1964.
9. Jansson E, Wager O, Forsvall P & Sternstrom R. Epidemic occurrence of adenovirus type 7 infection in Helsinki. *Ann Paediatr Fenn* 8: 24 1962.
10. Jen J, P. Tai Y, Lin Y C & Wang H Y. The role of adenovirus in the etiology of infantile pneumonia and pneumonia complicating measles. *Chin Med J (Peking)* 81: 141 1962.
11. Kunk U & Joopila R. Acute cerebellar ataxia associated with adenovirus type 7 infection. To be published.

haemoglobin was less than 10 g/100 ml also in two patients. The blood leucocytes were less than 5000 per mm<sup>3</sup> blood in the acute phase of the disease in three patients while in others the leucocyte counts were normal or slightly increased. Three patients showed neutrophilia. No monocytosis was noted. The blood thrombocytes were less than 100 000 in eight patients, but less than 50 000 in only one patient.

### EEG findings

In four patients in the acute phase of the disease examined with electroencephalography pathological findings comparable with encephalitis (8) were recorded (Table 2). A follow up EEG was carried out on two patients in the convalescent phase: in one the finding was normal while in the other the changes had diminished compared with the first examination.

### Prognosis

In three cases the disease was fatal. In one case the encephalitis was the direct cause of death while in two the disease was complicated by severe thrombocytopenic haemorrhagic diathesis. Treatment with prednisolone was attempted but with no observable effect on thrombocytopenic or haemorrhagic diathesis. Autopsy was carried out on all three and the findings agreed with an adenovirus infection. The histological examination of the brain revealed lymphocyte infiltration in one patient and only oedema and diffuse cellular changes in two. The most striking findings were in the lungs which were heavy, solid in consistency and dark red in colour. Microscopically the predominant feature was a diffuse interstitial pneumonitis, characterized by a moderate mononuclear cell infiltrate and hyperplasia of the alveolar lining cells. Many of these alveolar cells were greatly enlarged with large nuclei containing one or multiple eosinophilic inclusions. This picture is similar to that of fatal infections associated with adenovirus type 7 reported in literature (3, 15, 17, 20).

All patients were treated with antibiotics. They were hospitalized for 3-48 days, five pa-

tients for 3-14 days, two for 14-21 days, one more than 21 days.

### DISCUSSION

Pharyngoconjunctival fever in children and a mild infection of the upper respiratory tract in adults are usually considered the most typical clinical manifestations of an adenovirus infection. According to the present observations it would seem however that adenovirus type 7 may in early infancy produce a very severe clinical picture. Encephalomeningitis was unexpectedly frequent in this age group. Of the 32 children with adenovirus infection treated in our hospital, no less than seven showed the clinical picture of encephalomeningitis and one that of meningitis. It seems that encephalomeningitis is often associated with the adenovirus type 7 infection particularly in the second and third year of age. School age children have neurologic symptoms distinctly less often in connection with this virus infection (9). In these cases the clinical picture seems to be one of the meningitis (9). In older children adenovirus type 7 may cause isolated cerebellar ataxia (11), myo- or pericarditis (2, 4, 25).

The special clinical symptoms which may occur in infection caused by adenovirus type 7 are considerable oedema, hepatomegaly and in the most severe cases thrombocytopenic haemorrhagic diathesis. These symptoms at present suggest a very severe infection (1). Leucocytosis and neutrophilia may belong to the clinical picture (9, 23) as may monocytosis (6, 9). Adenovirus infection has often been found to follow a bacterial or viral infection (10, 17, 19), especially measles. In two of the present patients with encephalomeningitis some other virus infection (measles and chickenpox) preceded the adenovirus infection. None of the present patients however revealed any immunologic defect. The serum electrophoreses and immunoglobulins were normal in all cases.

The extent of the epidemic in the province was not known and therefore the rate of encephalomeningitis calculated from total morbidity

## JUVENILE HYPOTHYROIDISM WITH TESTICULAR ENLARGEMENT

Z. LARON, M. KARP and L. DOLBERG

*From the Paediatric Metabolic and Endocrine Service and the Pathological Institute  
Beckman Medical Center Petah Tikva, Israel*

Though hypothyroidism is one of the more frequently encountered endocrine disorders in the paediatric age group (14) little documentation has been made of the development of the gonads when thyroxine secretion is inadequate. Longstanding prepubertal hypothyroidism has been described as resulting in delayed maturation of the testicles in boys (1-8) and delayed puberty in girls (7, 12). According to these investigators this condition which was accompanied by decreased excretion of urinary gonadotrophins and sex hormones was corrected by treatment with thyroid preparations.

In contrast to the latter clinical picture hypothyroidism has also been known to be associated with precocious sexual development. This condition was first reported by Kendle (6) in 1905 but it was only in 1955 that a report by Bergstrand (2) drew renewed attention to it. It is noteworthy that in a total of 17 cases reported since then from various countries (4, 5, 9, 10, 13) only 3 have been males (in one of these the hypothyroidism was associated with Down's syndrome (9)).

In the present paper we report on four more male patients with hypothyroidism and testicular enlargement.

Pregnancy and delivery were normal. From the age of 3 years the parents had noticed obesity, sluggishness and a decreased growth rate but he did not receive any treatment.



*Fig. 1. Patient M. Y. at the age of 6 1/2 years. Note short stature for age, plumpness and large testicles without pubic hair.*

### CASE REPORTS

*Patient 1.* M. Y. was referred at the age of 6 1/2 years because of growth and mental retardation. Both parents and three other children were healthy and there was no family history of thyroid disease.

- 12 Kennedy C & Wanglee P Encephalitis A variable syndrome in response to viral infection *Pediat Clin N Amer* 14 809 1967
- 13 Kibrick S Meléndez L & Enders F J Clinical associations of enteric viruses with particular reference to agents exhibiting properties of the ECHO group *Ann NY Acad Sci* 67 311 1957
- 14 Käckell M Y Encephalomyelitis infolge Adenovirus Typ-3 Infektion *Mtschr Kinderheilk* 112 99 1964
- 15 Lelong M Léprie P Alison F Le Tan Vinh Saigé P & Chany C La pneumonie a virus du groupe A P C chez le nourisson isolément du virus *Lesi Meurs anatomohistologiques Arch Franc Pediat* 13 1092 1956
- 16 Mantylä R Adenovirus infections in servicemen in Finland *Ann Med Exp Biol Fenn* 44 Suppl 4 1966
- 17 Nahmas A J Griffin D & Sautzer J Fatal pneumonia associated with adenovirus type 7 *Amer J Dis Child* 114 36 1967
- 18 Osvath P & Toth M Eine durch Adenovirus 7 verursachte Kindergarten Epidemie *Arch Kinderheilk* 172 286 1965
- 19 Patzer von H & Braun W Die Adenovirus pneumonie Symptomatologie und Klinik *Munchen Med Wschr* 107 1985 1965
- 20 Sattellau G Der Nachweis einer Adenovirusinfektion (Typ 3) bei schwer bzw tödlich verlaufender Meningo Enzephalitis *Arch Kinderheilk* 170 174 1965
- 21 Sever J L Application of a microtechnique to viral serological investigations *J Immun* 85 379 1962
- 22 Solier R Chardonnet Y & Prunieras M Adenoviruses Status of current knowledge *Prog Med Virol* 7 253 1965
- 23 Spreer F Tauschnitz Ch Schmidt L Schmitz J Keil R & Cotteschling C Tödliche Adenoviruspneumonie im Kleinkindalter Bericht über zwei eigene Beobachtungen *Arch Kinderheilk* 172 284 1965
- 24 Stenger K Haffanke V & Schaeferhammer G Über eine Adenovirus Epidemie mit schwerer Encephalopathie *Mtschr Kinderheilk* 111 63 1965
- 25 Van Zaase D J & Van der Veer J Some peculiar clinical symptoms in children with adenovirus infection *Presse Med* 70 1071 1967

Submitted May 3 1969

Accepted Nov 7 1969

(S S) Dept of Paediatrics  
University of Oulu  
Oulu  
Finland

**Key words** Adenovirus type 7 infection viral encephalomyelitis

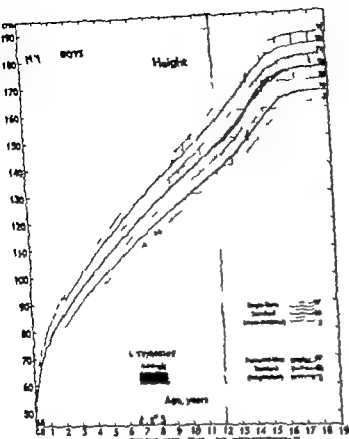


Fig 4 Patient X Y Growth chart denoting good response of linear height to thyroid therapy

one year of treatment there was no change in the testicular volume. The child is mentally retarded and attends a special school.

**Patient 2 J S** was referred to us at the age of 14 years because of mental retardation. There was no family history of thyroid disease. His motor development was slow and at the age of 3 years he had been diagnosed as suffering from hypothyroidism. He was subsequently treated irregularly with thyroid preparations until 6 months before he came to us. During these 6 months he had become obese, was unable to walk and constipated. Physical examination: non-reverted, mentally retarded boy. The skin was pale and cold. There was bilateral pretibial myxoedema. The thyroid gland was not palpable. His height 144 cm corresponding to a height age of 12 years. The skeletal age was 12 years. A blood count was normal but pulse rate was normal for his age. The penis was of normal size but the testis were enlarged with a volume of 15 ml each. Laboratory data revealed: Hemoglobin 7 g/100 ml erythrocytes 3.2  $10^6$ /mm<sup>3</sup>, cholesterol 4.5 mg/100 ml, PBI 2.9  $\mu$ g/100 ml, Thyroid radioiodine uptake (table) was 6.5% after 4 hours and did not change after TSH injection (400 IU). Urinary 17 ketosteroids were 1.5 mg/24 hours and the 17 hydroxycorticosteroids 4.4 mg/4 hours. The

seropituitary and ACTH tests were normal. Urinary gonadotrophins were 3 MIU/24 hours. X-ray of the skull showed an enlarged sella.

During 5 years of treatment with thyroid extract or l-thyroxine the boy achieved normal height (168 cm at the age of 20 years) and a normal sexual development. There was a reduction in the size of the testes and at the age of 20 years their volume was 20 ml which is within the normal range for this age. Testicular biopsy was not performed. The young man is mentally retarded and institutionalized.

**Patient 3 A W** was referred to us at the age of 17 years because of mental retardation. Both parents and two brothers aged 18 and 22 years are healthy. Another younger brother is described as case 4. The parents claim that the boy had already shown signs of motor and mental retardation in early infancy.

Physical examination revealed a boy with a proportional body build. Body height was 160 cm corresponding to a height age of 14 years. His epiphyse were closed. He had no goiter.

Pubic and axillary hair were of normal appearance. The penis length was 9.5 cm but the testes were abnormally large with a volume of over 50 ml each. X-ray of the skull was normal. Laboratory findings were: PBI 5.8  $\mu$ g/100 ml, BBT 2.5  $\mu$ g/

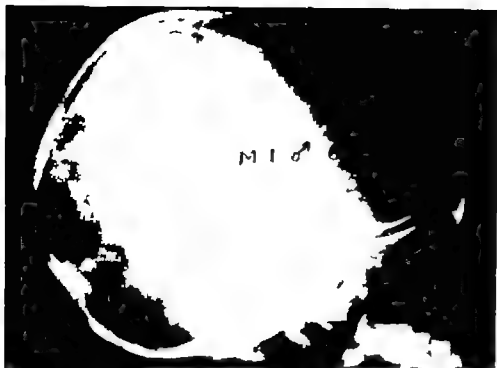


Fig 2 Patient M.Y.  
Roentgenogram of the  
skull showing an enlarged  
sella turcica

Physical examination revealed a pale, moderately obese child (Fig 1). The skin was dry and dental development was retarded. The thyroid gland was not palpable. Linear height was 103.5 cm, corresponding to a height age of 4  $\frac{1}{2}$  years. The skeletal age was 1 year. Pubic and axillary hair were absent. Penile length was 3.3 cm. Both testes were firm and enlarged for his age (15), measuring 7.8 ml in volume (Fig 1). Laboratory data revealed Hemoglobin 10.3 g/100 ml, erythrocytes  $3 \times 10^6$ /mm<sup>3</sup>, cholesterol 280 mg/100 ml, PBI 18  $\mu$ g/100 ml. Thyroid radioactive <sup>131</sup>I uptake was 10.5% after 2 hours without a further rise after 24 hours nor after TSH administration 200 IU in 2 injections. Urinary 17 ketosteroids were 0.4 mg/24 hours and 17 hydroxycorticosteroids 2.3 mg/24 hours. No gonadotropins were detected by bioassay in several 48-hour urine collections. Plasma gonadotropins were as fol-

lows: FSH—24.2 ng/ml (normal adult value  $17.4 \pm 3.8$  ng/ml), LH—1.5 ng/ml (normal adult value  $> 1.68$  ng/ml). (3) X-ray of the skull showed an enlarged sella (Fig 2). Biopsy of the testes was performed under general anesthesia. Histological examination revealed beginning spermatogenesis with a few spermatocytes and spermatids and a few Leydig cells (Fig 3), a picture consistent with that of beginning puberty.

Following therapy with l-thyroxine in a dose of 0.05 mg/day, there was a rapid advancement in growth velocity (Fig 4) and in skeletal age. The child became more active and lost weight. Dense-

Plasma gonadotropins were kindly measured for us by Dr P. Franchimont, Department of Clinical Medicine and Medical Pathology, University of Leuven, Belgium, by a radioimmunoassay method.

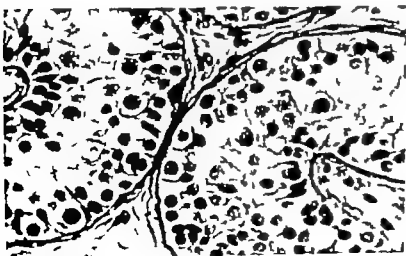


Fig 3 Patient M.Y. Histological  
appearance of testicular biopsy at age  
6  $\frac{1}{2}$ . The early spermatogenesis and  
Leydig cells are consistent with early  
puberty. HE—40.

not certain that he took his medication. His height and secondary sexual development progressed normally. Testicular biopsy performed at the age of 15  $\frac{1}{2}$  years showed a normal histological picture for this age.

A summary of the pertinent clinical data of the 4 patients and the other 3 male patients reported in the literature are presented in Table 1.

## DISCUSSION

It has yet to be clarified why primary hypothyroidism which remains untreated for long periods of time should affect sexual maturation in opposite ways, i.e. it may result in a lack of sexual maturation and a failure of sexual organs to develop or in precocious puberty and an abnormal increase in the size of the gonads. The latter phenomenon is more frequently encountered in girls.

Van Wyk & Grumbach (13) suggested that the precocious puberty might have resulted from an overlap mechanism of the hormones TSH and the gonadotrophins or their releasing factors at the hypothalamic or pituitary level. Among our 4 patients only in patient 1 were we able to prove the existence of elevated plasma FSH and LH levels (measured by radioimmunoassay) in the absence of urinary gonadotrophins (measured by bioassay). The other patients were too old to draw any conclusions from the moderately increased gonadotrophin output found in the urinary assay. Even so the finding of increased plasma FSH and LH in the one patient tested fails to indicate the exact mechanism involved. The enlarged sella turcica found in several of the patients could have resulted from hypertrophy of the thyrotrophin cells alone.

It is noteworthy that the most common manifestation of precocious sexual development in boys with hypothyroidism is an enlargement of the testicles. This is not particularly surprising since enlargement of the testes is the first sign of puberty in boys (11). Considering the fact that in the older patients there was a marked increase in the size of the testes, but no signs of increased virilization nor any increase in the urinary 17 ketosteroid excretion

it can be assumed that the secretion of FSH is much greater than that of ICSH. Further evidence supporting this assumption is the histological appearance of these testes which showed a predominance of tubular development, but no increase in the number of Leydig cells. Unfortunately it was not possible to perform plasma gonadotrophin determinations in the remaining three patients. The absence of an advanced bone age in these boys with precocious puberty may be due not only to the deficiency in androgens but to the primary deficiency of thyroxine which is necessary for normal bone growth and maturation.

It is noteworthy that De La Balze *et al* (1) reported an enlargement of testes in 3 of 6 patients with hypothyroidism who presented with delayed puberty. In these patients however the histological picture was abnormal showing edema of the interstitial tissue and thus differing from that seen in our patients. Furthermore the 3 older patients also had sexual hair and urinary gonadotrophins. However the observation made by De La Balze *et al* proves that in cases of primary hypothyroidism the existence of enlarged testes alone should not be taken as evidence for precocious sexual development. The presence of other secondary sexual characteristics, maturation of the seminiferous cells of the testes as shown by biopsy and an increase in serum gonadotrophins must all be confirmed before this diagnosis can be made.

There is no data available as to the correlation between the aetiology of hypothyroidism its duration before institution of treatment and the appearance of precocious signs of sexual development. All of our patients had had varying degrees of hypothyroidism from infancy. Three received no treatment and one probably received inadequate and irregular medication. It is interesting that two of these were brothers and had a metabolic type of hypothyroidism. These patients 3 and 4 had only a slight growth retardation and in patient 4 the skeletal retardation was less than 3 years. This can be assumed from an incomplete enzymatic de-



100 ml Thyroid radioactive  $^{125}\text{I}$  uptake was 18% after 2 hours and 38% after 24 hours  $^{125}\text{I}$  chromatography (150  $\mu\text{ci}$ ) revealed a conversion ratio of 4.5. After 24 hours there was 62% of the T<sub>3</sub>



Fig. 5 Patient A.W. at the age of 19 years. Note markedly enlarged testicles: volume > 50 ml

in the plasma. This amount disappeared after 2 hours. Large amounts of free iodine were found in the urine during 24 hours. These findings are consistent with hypothyroidism due to an enzymatic defect in the synthesis of thyroxine.

Within 2 years of treatment with a thyroid extract or with L-thyroxine there was no change in height, mental state or testicular volume (Fig. 5). Testicular biopsy performed at the age of 19 years revealed a histological structure compatible with that found in adulthood.

Patient 4 H.W., a brother of A.W., was first examined at the age of 12  $\frac{1}{2}$  years. Like his brother, this patient had shown signs of motor and mental retardation since early infancy. Physical examination showed a boy of proportional body build with a slow, sluggish speech. His height was 135 cm, corresponding to a bone age of 10 years. His bone age was 10 years. Axillary hair was absent and only fuzzy hair was found in the pubic area. The penis length was 5.3 cm and the testes were large for his age, measuring 15 ml in volume.

Laboratory findings were: PBI 50  $\mu\text{g}/100$  ml, BEI 28  $\mu\text{g}/100$  ml. Thyroid radioactive iodine uptake was 21% after 2 hours and 41% after 24 hours.  $^{125}\text{I}$  chromatography (100  $\mu\text{ci}$ ) showed a conversion ratio of 10%, with the same percentage distribution of T<sub>3</sub> and T<sub>4</sub> in the plasma as in his brother (patient 3). Urinary 17 ketosteroids were 3.2 mg/24 hours. Urinary gonadotropins were 4 MIU/24 hours. X-ray of the skull and the EEG were normal.

The boy received no treatment within the age year during which time there was a further development in the secondary sexual characteristics and an increase in the size of the testes. The volume of which reached 18 ml. Even after treatment with L-thyroxine was begun, the testes continued to increase in size, reaching a volume of 25–30 ml, but we are

Table 1 Pertinent data on male patients with hypothyroidism and precocious sexual development

Authors and references	Age at which onset of hypothyroidism was noted, years	Age at first examination, years	Plasma PBI $\mu\text{g}/100$ ml	RAI <sup>125</sup> I uptake at 24 hours	TSH reserve test	Pathologic plasma $^{125}\text{I}$ chromatography	Enlarged testicles	Testicular biopsy	Virilization	Urinary gonadotropins MIU/24 hrs	Plasma FSH	Plasma LH	Enlarged scrotum by X-ray	Reversal of symptoms on therapy
Franks & Stampfel (4)	5	7 <sup>†</sup>	3.2	4	-		+	P	-	-			-	+
Hubble		25					+		-	-				
Aaronson et al. (9) <sup>a</sup>							+		+					
Present report														
Pt. no. 1 M.Y.	2	6 <sup>†</sup>	1.8	10.5	-		+	P	-	-	†	†	+	-
Pt. no. 2 J.S.	3	14	2.3	4.5	-		+		-	3			+	+
Pt. no. 3 A.W.	1	17	5.8	38		+	+	N	-	6			-	-
Pt. no. 4 H.W.	1	12 <sup>†</sup>	5.0	41		+	+	N	-	4				

+ = Positive - = Negative † = Increased N = Normal P = Precocious maturation  
<sup>a</sup> = A case associated with Down's syndrome

not certain that he took his medication. His height and secondary sexual development progressed normally. Testicular biopsy performed at the age of 15 1/2 years showed a normal histological picture for that age.

A summary of the pertinent clinical data of the 4 patients and the other 3 male patients reported in the literature are presented in Table 1.

## DISCUSSION

It has yet to be clarified why primary hypothyroidism which remains untreated for long periods of time should affect sexual maturation in opposite ways, i.e. it may result in a lack of sexual maturation and a failure of sexual organs to develop or in precocious puberty and an abnormal increase in the size of the gonads. The latter phenomenon is more frequently encountered in girls.

Van Wyk & Grumbach (13) suggested that the precocious puberty might have resulted from an overlap mechanism of the hormones TSH and the gonadotrophins or their releasing factors at the hypothalamic or pituitary level. Among our 4 patients only in patient 1 were we able to prove the existence of elevated plasma FSH and LH levels (measured by radioimmunoassay) in the absence of urinary gonadotrophins (measured by bioassay). The other patients were too old to draw any conclusions from the moderately increased gonadotrophin output found in the urinary assay. Even so the finding of increased plasma FSH and LH in the one patient tested fails to indicate the exact mechanism involved. The enlarged sella turcica found in several of the patients could have resulted from hypertrophy of the thyrotrophin cells alone.

It is noteworthy that the most common manifestation of precocious sexual development in boys with hypothyroidism is an enlargement of the testicles. This is not particularly surprising since enlargement of the testes is the first sign of puberty in boys (11). Considering the fact that in the older patients there was a marked increase in the size of the testes but no signs of increased virilization nor any increase in the urinary 17 ketosteroid excretion

it can be assumed that the secretion of FSH is much greater than that of ICSH. Further evidence supporting this assumption is the histological appearance of these testes which showed a predominance of tubular development but no increase in the number of Leydig cells. Unfortunately it was not possible to perform plasma gonadotrophin determinations in the remaining three patients. The absence of an advanced bone age in these boys with precocious puberty may be due not only to the deficiency in androgens but to the primary deficiency of thyroxine which is necessary for normal bone growth and maturation.

It is noteworthy that De La Balze *et al* (1) reported an enlargement of testes in 3 of 6 patients with hypothyroidism who presented with delayed puberty. In these patients however the histological picture was abnormal showing edema of the interstitial tissue and thus differing from that seen in our patients. Furthermore the 3 older patients also had sexual hair and urinary gonadotrophins. However the observation made by De La Balze *et al* proves that in cases of primary hypothyroidism the existence of enlarged testes alone should not be taken as evidence for precocious sexual development. The presence of other secondary sexual characteristics, maturation of the seminiferous cells of the testes as shown by biopsy and an increase in serum gonadotrophins must all be confirmed before this diagnosis can be made.

There is no data available as to the correlation between the aetiology of hypothyroidism, its duration before institution of treatment and the appearance of precocious signs of sexual development. All of our patients had had varying degrees of hypothyroidism from infancy. Three received no treatment and one probably received inadequate and irregular medication. It is interesting that two of these were brothers and had a metabolic type of hypothyroidism. These patients 3 and 4 had only a slight growth retardation and in patient 4 the skeletal retardation was less than 3 years. This can be assumed from an incomplete enzymatic de-

fect in the thyroid leading to progressing thyroxin insufficiency with increasing age and enlarging body mass. To the best of our knowledge this is the first description of this kind of hypothyroidism associated with precocious sexual development.

SUMMARY

Four boys with primary hypothyroidism and enlarged testicles are described. The patients were studied at the ages of 6<sup>2</sup>/<sub>1</sub>, 12<sup>2</sup>/<sub>1</sub>, 14<sup>2</sup>/<sub>1</sub> and 17<sup>2</sup>/<sub>1</sub> years. Two had hypothyroidism of unknown etiology. The other two who were brothers, suffered from an enzymatic error in thyroxine synthesis. The common features were precocious and abnormal enlargement of the testicles. In the youngest patient precocious maturation of the seminiferous cells was proved by testicular biopsy. It is assumed that the aetiology of this syndrome is a hormonal overlap between TSH and gonadotrophins, a hypothesis which is favored by the finding of increased plasma gonadotrophins in the only patient tested. The marked enlargement of the testicles without any excessive virilization is assumed to result from a greater secretion of FSH than of ICSH.

ACKNOWLEDGEMENT

The authors wish to express their thanks to Prof Dr E. Tonutti of the Abteilung für Klinische Morphologie Universität Ulm for having so kindly reviewed the biopsy specimens and to Dr M. Dintzman and Dr C. Chumoff of the Department of Surgery A Beilinson Hospital for having made the biopsies.

REFERENCES

1 Balzo D, La F A, Arriaga F, Mancini R, E Janches M, Davidson O, W C, Gurtman A I. Male hypogonadism in hypothyroidism. *J Clin Endocr* 22: 217 1962.

2 Bergstrand C G. A case of hypothyroidism with signs of precocious sexual development. *Acta Endocr* 20: 338 1955.

3 Franchimont P. Personal communication.

4 Franks R C & Stempfel R S. Juvenile hypothyroidism and precocious testicular maturation. *J Clin Endocr* 23: 805 1963.

5 Hubble D. Endocrine relations. *Lancet* 11: 1965.

6 Kendle F W. Case of precocious puberty in a female child. *Brit Med J* 1: 246 1905.

7 Lederer J. *Les relations thyro-ovariennes*. Masson Paris 1946.

8 Lederer J, Roger F & Bataille J P. *Le mécanisme de l'insuffisance génitale masculine dans l'hypothyroïdisme primaire*. Actualités Endocrinologiques (La Pléiade). L'Expansion Paris 1967 p 231.

9 Maranon G, Martinez, D J & Gocho M J V. Mongolism et Macrogonosomie. *Ann Endocr (Paris)* 23: 41 1951.

10 Pabu H F, Prieschel S & Hillman D A. Etiologic interrelationship in Down's syndrome. Hypothyroidism and precocious sexual development. *Pediatrics* 40: 590 1967.

11 Tanner J M. *Growth at Adolescence*. Blackwell Scientific Publ. Oxford 1962. 2nd ed.

12 Vigot J & Favier G. Les retards pubertaires chez la fille. *Rev Prat (Paris)* 44: 109 1958.

13 Van Wyk J J & Grumbach M M. Syndrome of precocious menstruation and galactorrhea in juvenile hypothyroidism. An example of hormonal overlap in pituitary feedback. *J Pediatr* 57: 416 1960.

14 Williams L. *The diagnosis and treatment of endocrine disorders in childhood and adolescence*. Charles C Thomas Springfield Ill 1965. 3rd ed. pp 7 and 230.

15 Zifla E & Laron Z. The normal testicular volume in Israeli children and adolescents. *Harefuah* 77: 511 1969.

Submitted May 10 1969  
Accepted Nov 4 1969

(Z. L.) Paediatric Metabolic and Endocrine Service  
Beilinson Hospital  
P.O.B. 85  
Petah Tikva  
Israel

Key words: Juvenile hypothyroidism; testicular enlargement; precocious puberty.

## ATAXIC SYNDROME IN CONGENITAL HYPOTHYROIDISM

BENGT HAGBERG and OTTO WESTPHAL

*From the Department of Paediatrics, University Hospital, Uppsala, Sweden*

The adverse effect on the developing brain of an intra- or extruterine atthyroid or hypothyroid state is well known. The classical result is mental retardation of a variable degree. Behavioral deviations and abnormalities recorded at electroencephalographic examination (1, 17) have also been noted but neurological syndromes with motor handicaps are only seldom reported.

In a follow-up study comprising all cases of ataxia in infancy and childhood from the Uppsala area during the years 1956-1961 one of the authors (6) had two mildly ataxic patients with congenital hypothyroidism one of them adequately substituted since the age of 8 weeks. Since this preliminary observation all cases in Uppsala with the diagnosis of congenital hypothyroidism have been particularly observed for the presence or absence of ataxic signs. This paper presents our experiences from a small but unselected clinical series collected during a 12 year period.

### CLINICAL MATERIAL

In the area of Uppsala with about 180 000 inhabitants 11 children with congenital hypothyroidism were diagnosed in 1956-1969. In addition one child (5 L.) referred to the hospital from another region. Out of about three patients are summarized in Table 1. The first (case 1) was started as soon as the diagnosis was proved either dequated thyroid or sodium thyrone being used. In all cases the dose was kept at the highest tolerat. The treatment was considered off and when evidence of normal skeletal matu-

tion, normal growth development and normal PBI and cholesterol was observed. Physical motor development, opentical and neurological examinations were performed regularly.

### RESULTS

The results of these examinations are summarized in Table 1. Ataxia of the cerebellar type was found in 6 of the 12 patients and three of them are described in some detail below.

#### Case 1

T B record no UAS 103/61. This boy born February 12 1955 is the eldest child of three in a healthy family. The delivery was uneventful 7 weeks before term and the birth weight 3470 g. Because of vomiting and moderate neonatal icterus (upper level at 160  $\mu$ g/100 ml on the 11th day) he was kept in the pediatric clinic during the neonatal period but no abnormal neurological signs were observed. It was 6 weeks before his icterus had completely disappeared.

When 10 1 months old he was again sent for consultation because of late psychomotor development (not able to sit or creep) and a myxedematous appearance. PBI was 1.6 microgram/100 ml and cholesterol 30  $\mu$ g/100 ml. The neurological findings were considered normal except for a convergent squint. He was put on a relatively high dose of thyroid which he has since been receiving in a well controlled dosage.

He walked when 25 months old but was early considered to be a very clumsy child, unstable for his age and retarded in his speech development. When re-examined neurologically at 4 years of age his way of running was found to be abnormal and dysharmonious and he had a slight but evident cerebellar ataxia with intention tremor and retarded speech.

When 6 years old he was observed for 1 week at

fect in the thyroid leading to progressing thyroxine insufficiency with increasing age and enlarging body mass. To the best of our knowledge this is the first description of this kind of hypothyroidism associated with precocious sexual development.

### SUMMARY

Four boys with primary hypothyroidism and enlarged testicles are described. The patients were studied at the ages of 6 $\frac{1}{2}$ , 12 $\frac{1}{2}$ , 14 $\frac{1}{2}$ , and 17 $\frac{1}{2}$  years. Two had hypothyroidism of unknown etiology. The other two who were brothers suffered from an enzymatic error in thyroxine synthesis. The common features were precocious and abnormal enlargement of the testicles. In the youngest patient precocious maturation of the seminiferous cells was proved by testicular biopsy. It is assumed that the etiology of this syndrome is a hormonal overlap between TSH and gonadotrophins, a hypothesis which is favored by the finding of increased plasma gonadotrophins in the only patient tested. The marked enlargement of the testicles without any excessive virilization is assumed to result from a greater secretion of FSH than of ICSH.

### ACKNOWLEDGEMENT

The authors wish to express their thanks to Prof. Dr. E. Tonutti of the Abteilung für Klinische Morphologie, Universität Ulm, for having so kindly reviewed the biopsy specimens, and to Dr. M. Dintzman and Dr. C. Chaumoff of the Department of Surgery, A. Beilinson Hospital, for having made the biopsies.

### REFERENCES

- 1 Balze De La F. A., Arriaga F., Mancini R. E., Janches M., Davidson O. W. & Gurtman A. I. Male hypogonadism in hypothyroidism. *J. Clin. Endocr.* 22: 212, 1962.

- 2 Bergstrand C. G. A case of hypothyroidism with signs of precocious sexual development. *Acta Endocr.* 20: 338, 1955.
- 3 Franchimont P. Personal communication.
- 4 Franks R. C. & Stimpfel R. S. Juvenile hypothyroidism and precocious testicular maturation. *J. Clin. Endocr.* 23: 805, 1963.
- 5 Hubble D. Endocrine relations. *Lancet* 1: 1965.
- 6 Kendle F. W. Case of precocious puberty in a female child. *Brit. Med. J.* 1: 246, 1905.
- 7 Lederer J. *Les relations thyro-maternelles*. Masson, Paris, 1946.
- 8 Lederer J., Roger F. & Bataille J. P. *Le mécanisme de l'insuffisance génitale masculine dans l'hypothyroïdisme précoce*. Actualités Endocrinologiques (La Presse), L'Expansion, Paris, 1967, p. 231.
- 9 Maranon G., Martinez, D. J. & Gocho M. J. V. Monogonism et Microgonosomie. *Ann. Endocr. (Paris)* 23: 41, 1951.
- 10 Pabst H. F., Puschel S. & Hillman D. A. Etiologic interrelationship in Down's syndrome: Hypothyroidism and precocious sexual development. *Pediatrics* 40: 590, 1967.
- 11 Tanner J. M. *Growth at Adolescence*. Blackwell Scientific Publ., Oxford, 1962, 2nd ed.
- 12 Vague J. & Favner G. Les retards pubertaires chez la fille. *Rev. Prat. (Paris)* 44: 109, 1958.
- 13 Van Wyk J. J. & Grumbach M. M. Syndrome of precocious menarche and galactorrhea in juvenile hypothyroidism: An example of hormonal overlap in pituitary feedback. *J. Pediatr.* 57: 416, 1960.
- 14 Wilkins L. *The diagnosis and treatment of endocrine disorders in childhood and adolescence*. Charles C. Thomas, Springfield, Ill., 1965, 3rd ed., pp. 7 and 230.
- 15 Zifka E. & Laron Z. The normal testicular volume in Israeli children and adolescents. *Harefush* 77: 511, 1969.

Submitted May 10, 1969

Accepted Nov. 4, 1969

(Z. L.) Pediatric Metabolic and Endocrine Service, Beilinson Hospital, P.O.B. 85, Petah Tikva, Israel.

**Key words:** Juvenile hypothyroidism, testicular enlargement, precocious puberty.

## ATAXIC SYNDROME IN CONGENITAL HYPOTHYROIDISM

BENGT HAGBERG and OTTO WESTPHAL

From the Department of Paediatrics University Hospital Uppsala Sweden

The adverse effect on the developing brain of an intra or extrauterine at thyroid or hypothyroid state is well known. The classical result is mental retardation of a variable degree. Behavioral deviations and abnormalities recorded at electroencephalographic examination (1-17) have also been noted but neurological syndromes with motor handicaps are only seldom reported.

In a follow up study comprising all cases of ataxia in infancy and childhood from the Uppsala area during the years 1956-1961 one of the authors (6) had two mildly ataxic patients with congenital hypothyroidism one of them already quietly substituted since the age of 8 weeks. Since this preliminary observation all cases in Uppsala with the diagnosis of congenital hypothyroidism have been particularly observed for the presence or absence of ataxic signs. This paper presents our experiences from a small but unselected clinical series collected during a 12 year period.

### CLINICAL MATERIAL

In the area of Uppsala with about 180 000 inhabitants 11 children with congenital hypothyroidism were raised in 1956-1969. In addition one child (5 L.) was referred to the hospital from another region. Out of these patients are summarized in Table 1. The treatment was started as soon as the diagnosis was proved either by elevated thyroid or sodium thyroxine test used in all cases the dose was kept at the highest tolerated. The treatment was considered off when evidence of normal skeletal maturation

and normal growth development and normal PBI and cholesterol was observed. Physical motor development and neurological examinations were performed regularly.

### RESULTS

The results of these examinations are summarized in Table 1. Ataxia of the cerebellar type was found in 6 of the 12 patients and three of them are described in some detail below.

#### Case 1

T B record no 1145 103/61. This boy born February 12 1955 is the eldest child of three in a healthy family. The delivery was uneventful 2 weeks before term and the birth weight 3470 g. Because of vomiting and moderate neonatal icterus (upper level at 16.0 mg/100 ml on the 11th day) he was seen at the pediatric clinic during the neonatal period but no abnormal neurological signs were observed. It was 6 weeks before his mother had completely disappeared.

When 10 months old he was again sent for consultation because of late psychomotor development (not able to sit or creep) and a myxedematous appearance. PBI was 16 micrograms/100 ml and cholesterol 250 mg/100 ml. The neurological findings were considered normal except for a convergent strabismus. He was put on a relatively high dose of thyroid which he has since been receiving at a well controlled dosage.

He walked when 25 months old but was early considered to be a very clumsy child, inflexible for his age and retarded in his speech development. When re-examined neurologically at 4 1/2 years of age his way of running was found to be abnormal and dysrhythmic and he had a slight but evident cerebellar ataxia with intention tremor and retarded speech.

When 6 years old he was observed for 1 week at

Case	Date of birth	Sex	Hypothyroidism diagnosed age (months)	PBI ( $\mu$ g/100 ml)	Walked age (months)	Ataxia diagn. age (years)	Intel. level	Latest follow up age (years)	Running	Altering jumping	Intention tremor	Writing style	Pinpoint test
T B	550212	o	11	—	25	4½	IQ 50-60	14	Dysarthric monic	Dysarthric monic	+	Bad	Tremor
M A	640815	f	24	0.8	31	2½	IQ 80	5	Dysarthric monic	Impossible	++	—	Tremor
S L	620620	o	9	1.8	—	3	Imbecile	7	—	—	+++	—	—
L L	511019	o	10	—	25	8	IQ 64	16	Clumsy Normal	Impossible	+++	Bad	Tremor
M K	570314	o	15	1.4	24	4½	Low normal	12	Normal	Normal	+	Good	Tremor
A S	580309	f	2	2.5	19	3	IQ 94	11	Dysarthric monic	Dysarthric monic	—	Normal	Normal
T N	550530	o	6	—	16	—	Normal	6	Normal	Normal	—	—	—
L K	560406	o	3	1.7	19	—	IQ 63	13	Normal	Normal	—	—	—
I B	601229	o	6	2.5	25	—	Imbecile	7	Clumsy Normal	—	—	Good	Normal
E B	621016	o	10	2.2	16	—	Low/educable	5	Normal	Normal	—	—	—
N W	630305	o	1½	0.8	16	—	Normal	6	Normal	Normal	—	—	Normal
H G	660423	o	16	2.4	14	—	Normal	4	Normal	Normal	—	—	—

the pediatric clinic. He was found to be mentally retarded with an intelligence quotient of 48 according to Terman Merrill and 58 according to Buhler Rater. His ataxic signs of the cerebellar type were once again established. EEG was abnormal with numerous epileptogenic discharges but he had no convulsions. He was sent to a school for mentally retarded and has been there ever since.

In spite of intensive training during the following years he still had great difficulties in learning common motor performances. Thus he was 1½ years of age when he was first able to ride a two wheel bicycle after about 4 years of training and was still unable to skate at the age of 15 years. His fine motor dysfunction was less remarkable but was obvious in daily life and in his style of writing.

When last seen at 14 years of age he had obvious difficulties in buttoning his clothes tying his shoe laces and filling a glass without spilling. His gross motor function was fairly well compensated but his running was stiffer and slower than normal. Neurological examination still revealed a relatively well compensated cerebellar ataxia with intention tremor but no hyperkinesia and no signs of spasticity. In summary his ataxic abnormality had gradually become less evident in his daily life during the last few years.

#### Case 2

M A record no UAS 166/66. The first child of two in a healthy family this girl was born on August 15 1964 3 weeks before term. There were no complications during the pregnancy or delivery and the birth weight was 3200 g. Because of suspected dislocation of the hip the child was seen at the hospital at the age of 6 weeks. The hips were found to be normal but the child had a prolonged neonatal persistent icterus (bilirubin 7.2 mg/100 ml). Physical and neurological examination showed nothing abnormal. A blood sample for PBI was taken but for some reason was not analyzed. The family then moved to another part of the country.

It soon became obvious that the child's psychomotor development was retarded (she was able to sit at the age of 11 months and to creep at 1½ months but was not able to walk at 24 months). At the age of 24 months the child was admitted to a pediatric clinic. She exhibited a myxedematous appearance and her growth was considerably stunted. The bone age was considered to be about 8 months and the PBI was 0.8 microgram/100 ml. On motor age examination she showed a developmental level of about 11 months and muscular hypotonia particularly in the lower limbs. However no other neurological signs were noted. She was put on a fairly high dose of thyroid and since then the dosage has been kept at the upper tolerated limit.

She learned to walk at the age of 30 months but was found to be very clumsy. On re-examination when 3 years old an evident cerebellar ataxia with intention tremor and a very dysarthric way of

running were noted. Her speech was markedly retarded. Intensive motor training was started.

The girl was last seen at Uppsala at the age of 4 1/2 years and the cerebellar type ataxia was still evident. Her intention tremor was very obvious. She was unable to fill a glass without spilling or to unbutton her clothes. Her running was clumsy and slow and she was not able to jump in an adequate way for her size. No spastic signs were noted. She was found to have an intelligence quotient of 80 according to Terman Merrill.

To summarize this girl has a marked cerebellar ataxia now giving more problems in daily life than her moderate and uncomplicated mental retardation.

#### Case 3

*S. L. record no. UAS 1949/66.* This girl born on June 20 1942 is the second of three children. Her parents and siblings are all healthy. Pregnancy and delivery were uneventful. Her birth weight was 3770 g. She was said to be slightly icteric already at birth. Her ataxia was never severe and it disappeared after one week. Difficulties with feeding and general dullness were already observed during the neonatal period. Hypothyroidism was diagnosed at her paediatric county clinic when she was 9 months old mainly on the basis of her slow psychomotor development. Her PBI was found to be 1.8 micrograms/100 ml. Substitution therapy was immediately instituted.

The girl learned to roll over when 12 months old and to sit without support at 24 months. She was still markedly waddling and swaying when first seen at Uppsala at 3 1/2 years of age. She was also able then to shuffle along on her toes. Her motor age was found to be about 8 months at that stage. An obvious ataxia with slight atetoid movements of the trunk was observed.

When 4 1/2 years old she was seen for neurological consultation. She was still unable to walk and her motor age was found to be 10 months in the arms and hands and 8 months in the lower extremities. Neurological examination revealed a pronounced truncal ataxia with coarse truncal tremor and intention tremor in the arms and hands. In addition she had slight hyperkinetic movements of the atetoid type. There were no signs of spasticity. Her intellectual capacity was reduced. No formal testing was possible but it was judged that she functioned at an imbecile level. EEG was within normal limits on two different occasions. Air encephalography showed a moderate atrophic encephalopathy with generalized dilatation of the reticular system but no evidence of cortical atrophy.

At 7 years of age she was a little more steady in the sitting position but was unable to stand or walk. She could not eat nor dress herself and had no speech. Obviously the condition was unchanged in all essential respects. She was now being cared for in a county home for imbeciles and was considered to function at a relatively high imbecile level.

## DISCUSSION

Half of our 12 cases had ataxic signs of the cerebellar type with incoordination of movements and intention tremor but no objective evidence of components of dysequilibrium. In no case were signs of spasticity observed. The ataxia was nonprogressive rather showing a tendency towards compensation and functional improvement. Four children were only slightly affected and their condition might well have been overlooked had not a thorough history been taken and repeated neurological examinations performed. Their clumsiness and tendency to spill their drinks had on many occasions been attributed erroneously to mental dullness. The ataxia in one child was moderate in degree and one other child had a very pronounced and disabling motor handicap which in fact brought her to the clinic for neurological examination independent of her congenital hypothyroidism. It can always be argued that in this case the syndrome of cerebral palsy may be due to quite a different unknown prenatal lesion or malformation parallel to the congenital hypothyroidism. However in view of the similarity to the other five of our children this possibility seems fairly unlikely.

In older literature no ataxic but some diplegic syndromes have been reported in association with congenital hypothyroidism e.g. by McGarrison (14) and Neville (15) but their clinical description is consistent with the concept of spastic diplegia and no cerebellar signs seem to have been recorded.

In recent years some interesting observations concerning this subject have been made. In a review concerning the mental prognosis in 128 cases with hypothyroidism of infancy and childhood Smith and collaborators (18) found neurological signs such as shuffling gait incoordination awkwardness coarse tremor and spasticity in 26 of 79 patients with severe congenital hypothyroidism. No such signs were found in 32 cases with mild hypothyroidism. Another interesting point was that abnormal neurologic findings were noted in only 4 of 22



Table 1 Clinical material of congenital hypothyroidism admitted 1956-1969

Case	Date of birth	Sex	Hypothyroidism diagnosed age (months)	PBI ( $\mu$ g/100 ml)	Walked age (months)	Ataxia diagn. age (years)	Intel. level	Latest follow up age (years)	Running	Alter. nailing jump	Intention tremor	Writing style	Pinpoint test
T B	550212	♂	11	—	25	4½	IQ 50-60	14	Dysarthric monic	Dysarthric	+	Bad	Tremor
M A	640815	—	24	0.8	31	2½	IQ 80	5	Dysarthric monic	Impossible	++	—	Tremor
S L	620620	♀	9	1.8	—	3	Imbecile	7	Clumsy	Impossible	+++	—	—
L L	511019	♂	10	—	25	8	IQ 64	16	Normal	Normal	+++	Bad	Tremor
M K	570314	♂	15	1.4	24	4½	Low normal	12	Clumsy	Normal	+	Good	+
A S	580309	♀	2	2.5	19	3	IQ 94	11	Dysarthric monic	Dysarthric	—	Normal	Normal
T N	550530	♀	6	—	16	—	Normal	6	Normal	Normal	—	—	—
L N	560406	♂	3	1.7	19	—	IQ 63	11	Normal	Normal	—	Good	—
T B	601229	♂	6	2.5	25	—	Imbecile	7	Clumsy	Normal	—	Normal	—
E B	621016	♂	10	2.2	16	—	Low/educ	5	Normal	Normal	—	—	—
N W	630305	♂	1½	0.8	16	—	able	6	Normal	Normal	—	—	Normal
H O	660423	♂	16	2.4	14	—	Normal	4	Normal	Normal	—	—	—

the pediatric clinic. He was found to be mentally retarded with an intelligence quotient of 48 according to Terman Merrill and 58 according to Bahler-Heter. His ataxic signs of the cerebellar type were once again established. EEG was abnormal with numerous epileptogenic discharges but he had no convulsions. He was sent to a school for mentally retarded and has been there ever since.

In spite of intensive training during the following years he still had great difficulties in learning, common motor performances. Thus he was 12 years of age when he was first able to ride a two wheel bicycle after about 4 years of training and was still unable to skate at the age of 15 years. His fine motor dysfunction was less remarkable but was obvious in daily life and in his style of writing.

When last seen at 14 years of age he had obvious difficulties in buttoning his clothes, tying his shoe laces and filling a glass without spilling. His gross motor function was fairly well compensated but his running was stiffer and slower than normal. Neurological examination still revealed a relatively well compensated cerebellar ataxia with intention tremor but no hyperkinesia and no signs of spasticity. In summary his ataxic abnormality had gradually become less evident in his daily life during the last few years.

#### Case 2

*M A record no UAS 166/66* The first child of two in a healthy family this girl was born on August 15 1964 3 weeks before term. There were no complications during the pregnancy or delivery and the birth weight was 3200 g. Because of suspected dislocation of the hip the child was seen at the hospital at the age of 6 weeks. The hips were found to be normal but the child had a prolonged neonatal jaundice (bilirubin 7.2 mg/100 ml). Physical and neurological examination showed nothing abnormal. A blood sample for PBI was taken but for some reason was not analyzed. The family then moved to another part of the country.

It soon became obvious that the child's psychomotor development was retarded (she was able to sit at the age of 11 months and to creep at 12 months but was not able to walk at 24 months). At the age of 24 months the child was admitted to a pediatric clinic. She exhibited a myxedematous appearance and her growth was considerably stunted. The bone age was considered to be about 8 months and the PBI was 0.8 microgram/100 ml. On motor age examination she showed a developmental level of about 11 months and muscular hypotonia particularly in the lower limbs. However no other neurological signs were noted. She was put on a fairly high dose of thyroid and since then the dosage has been kept at the upper tolerated limit.

She learned to walk at the age of 30 months but was found to be very clumsy. On re-examination when 3 years old an evident cerebellar ataxia with intention tremor and a very dysarthric way of

running were noted. Her speech was markedly retarded. Intensive motor training was started.

The girl was last seen in Uppsala at the age 4½ years and the cerebellar type ataxia was still evident. Her intention tremor was very obvious. She was unable to fill a glass without spilling or to unbutton her clothes. Her running was clumsy and slow and she was not able to jump in an adequate way for her age. No spastic signs were noted. She was found to have an intelligence quotient of 80 according to Terman Merrill.

To summarize this girl has a marked cerebellar status now giving more problems in daily life than her moderate and uncomplicated mental retardation.

### Case 3

S. L. record no UAS 1949/66. This girl born in June 20 1961 is the second of three children. Her parents and siblings are all healthy. Pregnancy and delivery were uneventful. Her birth weight was 3220 g. She was said to be slightly icteric already at birth. Her icterus was never severe and it disappeared after one week. Difficulties with feeding and general distress were already observed during the neonatal period. Hypothyroidism was diagnosed at her pediatric county clinic when she was 9 months old mainly on the basis of her slow psychomotor development. Her PKU was found to be 1.8 microgram/100 ml. Substitutional therapy was immediately instituted.

The girl learned to roll over when 12 months old and to sit without support at 24 months. She was still markedly unsteady and swaying when first seen in Uppsala at 3½ years of age. She was also able then to shuffle along on her buttocks. Her motor age was found to be about 8 months at this stage. An obvious ataxia with slight athetoid movements of the hands was observed.

When 4 years old she was seen for neurological consultation. She was still unable to walk and her motor age was found to be 10 months in the arms and hands and 8 months in the lower extremities. Neurological examination revealed a pronounced truncal ataxia with coarse truncal tremor and intention tremor in the arms and hands. In addition she had slight hyperkinetic movements of the athetoid type. There were no signs of spasticity. Her intellectual capacity was reduced. No formal testing was possible but it was judged that she functioned at an umbilical level. EEG was within normal limits on two different occasions. Air encephalography showed a moderate atrophic coarctophathy with generalized dilatation of the ventricular system but no evidence of cortical atrophy.

At 7 years of age she was a little more steady in the sitting position but was unable to stand or walk. She could not eat nor dress herself and had no speech. Otherwise the condition was unchanged in all essential respects. She was now being cared for in a country home for umbilicals and was considered to function at a relatively high umbilical level.

## DISCUSSION

Half of our 12 cases had ataxic signs of the cerebellar type with incoordination of movements and intention tremor but no objective evidence of components of dysequilibrium. In no case were signs of spasticity observed. The ataxia was nonprogressive rather showing a tendency towards compensation and functional improvement. Four children were only slightly affected and their condition might well have been overlooked had not a thorough history been taken and repeated neurological examinations performed. Their clumsiness and tendency to spill their drinks had on many occasions been attributed erroneously to mental dullness. The ataxia in one child was moderate in degree and one other child had a very pronounced and disabling motor handicap which in fact brought her to the clinic for neurological examination independent of her congenital hypothyroidism. It can always be argued that in this case the syndrome of cerebral palsy may be due to quite a different unknown prenatal lesion or malformation parallel to the congenital hypothyroidism. However in view of the similarity to the other five of our children this possibility seems fairly unlikely.

In older literature no ataxic but some diplegic syndromes have been reported in association with congenital hypothyroidism e.g. by McGarrison (14) and Naville (15) but their clinical description is consistent with the concept of spastic diplegia and no cerebellar signs seem to have been recorded.

In recent years some interesting observations concerning this subject have been made. In a review concerning the mental prognosis in 128 cases with hypothyroidism of infancy and childhood Smith and collaborators (18) found neurological signs such as shuffling gait, incoordination, awkwardness, coarse tremor and spasticity in 26 of 79 patients with severe congenital hypothyroidism. No such signs were found in 32 cases with mild hypothyroidism. Another interesting point was that abnormal neurologic findings were noted in only 4 of 22

Table 1 Clinical material of congenital hypothyroidism admitted 1956-1969

Case	Date of birth	Sex	Hypothyroid diagnosed age (months)	PBI ( $\mu\text{g}/100\text{ ml}$ )	Walked age (months)	Ataxia diagn age (years)	Intel level	Latest follow up age (years)	Running	Alter nating jump	Inten tion tremor	Writing style	Pinpoint test
T B	540212	♂	11	—	25	4½	IQ 50	60	Dysar monic	Dysar monic	+	Bad	Tremor
M A	640815	+	24	0.8	31	2½	IQ 80	5	Dysar monic	Impos sible	++	—	Tremor
S L	620620	—	9	1.8	—	3	Imbecile	7	—	—	+++	—	—
L L	511019	♀	10	—	25	8	IQ 64	16	Clumsy	Impos sible	+++	Bad	Tremor
M K	570314	+	15	1.4	24	4½	Low normal	12	Normal	Normal	+	Good	Normal
A S	580309	+	2	2.5	19	3	IQ 94	11	Dysar monic	Dysar monic	—	Normal	Normal
T N	550530	♀	6	—	16	—	Normal	6	Normal	Normal	—	—	—
L K	560406	♀	3	1.7	19	—	IQ 63	13	Normal	Normal	—	Good	—
I B	601229	♀	6	2.5	25	—	Imbecile	7	Clumsy	—	—	Normal	Normal
E B	621016	♀	10	2.2	16	—	Low/educ able	5	Normal	—	—	—	—
N W	630405	♀	1½	0.8	16	—	Normal	6	Normal	Normal	—	—	Normal
H G	660423	♀	16	2.4	14	—	Normal	4	Normal	Normal	—	—	—

for age

the pediatric clinic. He was found to be mentally retarded with an intelligence quotient of 48 according to Terman Merrill and 58 according to Bühler-Hitzer. His ataxic signs of the cerebellar type were once again established. EEG was abnormal with some epileptogenic discharges but he had no convulsions. He was sent to a school for mentally retarded and has been there ever since.

In spite of intensive training during the following years he still had great difficulties in learning common motor performances. Thus he was 12 years of age when he was first able to ride a two-wheel bicycle after about 4 years of training and was still unable to skate at the age of 15 years. His fine motor dysfunction was less remarkable but was obvious in daily life and in his style of writing.

When first seen at 14 years of age he had obvious difficulties in buttoning his clothes, tying his shoe laces and filling a glass without spilling. His gross motor function was fairly well compensated but his running was slower and stiffer than normal. Neurological examination still revealed a relatively well compensated cerebellar ataxia with intention tremor but no hyperkinesia and no signs of spasticity. In summary his ataxic abnormality had gradually become less evident in his daily life during the last few years.

#### Case 2

*M A record no UAS 166/66* The first child of two in a healthy family, this girl was born on August 15 1964 3 weeks before term. There were no complications during the pregnancy or delivery and the birth weight was 3200 g. Because of suspected dislocation of the hip the child was seen at the hospital at the age of 6 weeks. The hips were found to be normal but the child had a prolonged neonatal persistent icterus (bilirubin 7.2 mg/100 ml). Physical and neurological examination showed nothing abnormal. A blood sample for PBI was taken but for some reason was not analyzed. The family then moved to another part of the country.

It soon became obvious that the child's psychomotor development was retarded (she was able to sit at the age of 11 months and to creep at 12 months but was not able to walk at 24 months). At the age of 24 months the child was admitted to a pediatric clinic. She exhibited a myxedematous appearance and her growth was considerably stunted. The bone age was considered to be about 8 months and the PBI was 0.8 microgram/100 ml. On motor age examination she showed a developmental level of about 11 months and muscular hypotonia particularly in the lower limbs. However no other neurological signs were noted. She was put on a fairly high dose of thyroid and since then the dosage has been kept at the upper tolerated limit.

She learned to walk at the age of 30 months but was found to be very clumsy. On re-examination when 3 years old an evident cerebellar ataxia with intention tremor and a very dysrhythmic way of

## ACKNOWLEDGEMENT

The investigation was supported by grants from Olle and Elof Ericson's foundation for scientific research, Folke Bernadotte's foundation for children with cerebral palsy and the Swedish medical research council project no. B68 19X 108-05A.

## REFERENCES

- 1 d'Agnon M & Malm L A. The electroencephalogram in congenital hypothyroidism. *Acta Paediatr Scand* 38 37 1949
- 2 Eayrs J T. The vascularity of the cerebral cortex in normal and cretinous rats. *J Anat* 88 164 1954
- 3 — The influence of the thyroid on the central nervous system. *Br Med Bull* 16 122 1960
- 4 Eayrs J T & Taylor S H. The effect of thyroid deficiency induced by methylthioacetate on the maturation of the central nervous system. *J Anat* 85 330 1951
- 5 Geloso J P, Henson P, Legrand J, Legrand C & Jost A. Some aspects of thyroid physiology during the perinatal period. *Gen Comp Endocr* 10 191 1965
- 6 Hagberg B. Ataxia in children. I. Survey and 5 years clinical material. *Läkarskriften* 59 1530 1962
- 7 Jellinek E H & Kelly R E. Cerebellar syndrome in myxoedema. *Lancet* ii 225 1960
- 8 Klonovskiy B M. *The development of the brain and its disturbances by harmful factors*. Pergamon Press Oxford London New York and Paris 1963 pp 152-161
- 9 Legrand J. Endocrinologie — Influence de l'hypothyroïdisme sur la maturation du cortex cérébral. *C R Acad Sci* 261 544 1965
- 10 — Analyse de l'action morphogénétique des hormones thyroïdiennes sur le cervelet du jeune rat. *Arch Anat Micro Morph Exp* 56 206 1967
- 11 — Variations en fonction de l'âge de la réponse du cervelet à l'action morphogénétique de la thyroïde chez le rat. *Arch Anat Micro Morph Exp* 56 291 1967
- 12 Legrand J, Knebel A & Jost A. Déficience thyroïdienne et maturation du cervelet chez le rat blanc. *Arch Anat Micro Morph Exp* 50 307 1961
- 13 Lotmar F. Entwicklungsstörungen in der Kleinhirnrinde beim endemischen Cretinismus. *Z Ges Neurol Psychiat* 136 412 1931
- 14 McGarrison R. *The thyroid gland in health and disease*. Wm Wood & Co Baltimore 1917 p 141
- 15 Neville F. Congenital diplegia and thyroid disturbance in abnormal infants of Geneva. *Schweiz Arch Neurol Neurochir Psychiat* 13 549 1923
- 16 Price T R & Neticky M G. Myxoedema and ataxia. Cerebellar alterations and neural myxoedema bodies. *Neurology* 16 957 1966
- 17 Schmitz M A, Schmitz F J & Parmelee A H. Schilddrüsenhormone und Hirnentwicklung. *Monat Kinderheilk* 113 284 1967
- 18 Smith D W, Blizard R M & Williams L. The mental prognosis in hypothyroidism of infancy and childhood. *Pediatrics* 19 1011 1957

Submitted Oct 6 1969

Accepted Nov 22 1969

(B H) Dept of Paediatrics  
Akademiska Sjukhuset  
750 14 Uppsala  
Sweden

Key words: Ataxia congenital hypothyroidism

patients treated within the first 6 months of life whereas 22 of 57 patients treated after 6 months of age showed such signs. Furthermore the degree of mental retardation was proportionate to the degree of neurologic impairment. In *Furmer's textbook Pediatric Neurology*, Jenkins in 1964 made the following statement: "spasticity, tremor, extrapyramidal rigidity, choreiform and athetotic movements, and optic nerve involvement may be seen." Klossovsky (8) mentions a group of 12 children, aged 2-11 years, insufficiently substituted with thyroid hormone; all twelve exhibited tremor of all extremities and three of them showed increased tendon reflexes and pyramidal signs.

Experimental studies on animals have confirmed the clinical and neuropathological evidence of the delay or arrest of brain development in man. Eayrs and his group (3, 4) observed several morphogenetic changes of the cerebral cortex in rat litters following artificially induced hypothyroidism at birth. Légrand & Jost and their collaborators (5, 9, 10, 11, 12) have made extensive corresponding studies on cerebellar cortex. They found that the histological maturation of the whole cerebellar cortex was altered and delayed in rats made hypothyroid through treatment with radioactive iodine and propylthiouracil before or shortly after birth (within the first two weeks). However, thyroidectomy was found ineffective when performed after postnatal day 14. It appears that cerebellar structures seem to be particularly dependent upon an adequate action of thyroid hormone during intrauterine, perinatal and early postnatal life. With all probability corresponding time schedules should also be valid on the whole for man, the length of the postnatal risk period being unknown, however. It is logical to believe that this period rather might truly be in months than in weeks and this is supported by the findings of Smith and collaborators (18).

It is of therapeutic interest to emphasize that thyroxine treatment when initiated before the end of the second postnatal week and not continued after that period was able to correct

all these alterations in the cerebellum of young rats. This constitutes yet further support for a particular and time limited stage of sensitivity to the hormone.

Interesting neuropathological observations supporting the validity of the animal studies also in man were made in adult cretin patients at an early date by Lotmar (13). Among other things, he found localized islands of fetal cerebellar cortex mixed in corresponding tissue of adult structure. He also revealed Purkinje cell changes, well in agreement with a cellular maturational arrest as found in the previously mentioned animal studies by Légrand *et al* (12).

To summarize, both experimental and neuropathological findings have provided a plausible explanation for the origin of ataxic syndromes in infants and children with congenital hypothyroidism not treated during their first months of life. From our cases it is of interest to note that case 6 was rather hyper than hyposubstituted with thyroid hormones from as early an age as 8 weeks and yet developed a mild but quite obvious ataxic syndrome. Thus the time limit for full prevention of cerebellar maturational derangement may be short also in the postnatal life of man.

## SUMMARY

Ataxia of the cerebellar type was found in six of twelve patients with congenital hypothyroidism admitted to the department of pediatrics of Uppsala during the years 1956-69. The median age for diagnosis and the start of adequate substitution therapy was 9-10 months, the youngest patient being 6 weeks and the eldest 24 months. The ataxia was non-progressive with a tendency towards compensation and functional improvement. Four cases were only slightly, one moderately and one severely disabled. Animal experiments and neuropathological observations in man supporting a cellular maturational arrest of cerebellar structures in congenital hypothyroid states are discussed.

[illegible]

REVIEW ARTICLE

BENIGN INTRACRANIAL HYPERTENSION  
(PSEUDOTUMOR CEREBRI)

*Review and Report of 18 Cases*

B HAGBERG and M SILLANPÄÄ

*From the Departments of Paediatrics University of Uppsala Uppsala Sweden and  
University of Turku Turku Finland*

Benign intracranial hypertension is one of several names given to a clinical syndrome of prolonged increased intracranial pressure, being due neither to a localized space process nor to the acute brain swelling effects of meningitis or encephalitis. It occurs in association with various etiologically known disorders such as sinus thrombosis and in some less well defined conditions such as para infectious and allergic states. There is also an idiopathic form (7-38). Synonymous names from the literature are pseudotumor cerebri, brain swelling of unknown cause, hypertensive meningeal hydrops, otitic hydrocephalus and toxic hydrocephalus. The condition was first described by Quincke in 1893 (62) under the designation of serous meningitis. Nonne introduced the name pseudotumor cerebri in 1914 (55). Davidoff & Dyle in 1936 (13) demonstrated that this syndrome occurred in the presence of a normal ventricular system. In most cases air encephalography or angiography must be performed in order to exclude the possibility of brain tumor. These patients usually complain of headache, vomiting, blurring of vision and diplopia. In cases with closed cranial sutures, papilledema is always noted and VIII nerve palsy and disturbed balance are some

times observed. Focal neurologic signs are only seldom encountered. In infants a bulging fontanel and diastasis of sutures may be the only signs. Lumbar or ventricular puncture reveals a normal composition of the cerebrospinal fluid (CSF) but an increased CSF pressure. This does not often affect the general condition, which is good in proportion to the magnitude of the signs of increased intracranial pressure.

As cases with the syndrome of pseudotumor cerebri often constitute large clinical difficulties for differential diagnosis and as our knowledge of the etiology and pathogenetic mechanisms of this condition are still incomplete we considered it worthwhile to summarize our own clinical experiences from 18 children, to give some characteristic case reports and to review the relevant literature.

OWN CLINICAL MATERIAL

During the years 1960-69, 18 infants and children in our clinics were diagnosed as having pseudotumor cerebri. This series is summarized in Table 1. The average age of the patients was 6 years and 7 months, a variation between 4 months and 19 years. There were 10 boys and 8 girls. Sixteen of the cases were diagnosed and treated in inpatient clinics and two in outpatient clinics. Some representative cases will be presented below.

normal with no normal background activity and diffuse symmetrical paroxysmal episodes. During the next month she deteriorated in spite of steroid treatment with 8 mg Dexamethasone<sup>1</sup> per day.

Because of increasing pain rapidly decreasing visual acuity nearly to blindness disc protrusions of 1 D and abundant leakage of CSF through the burr holes, bilateral subtemporal decompression was performed in September. Her general condition and vision thereafter improved. One month later her vision was 0.2 on the right eye and finger counts on the left. During the following month further improvement in vision and EEG were recorded.

In 1965 two years after the onset of her disease she had no headache and had passed her 4th school year without difficulties. Her temperament was said to be more labile than before the disease. Her Vth and VIth nerve pulses had completely disappeared. The vision was 0.7 on the right eye and 0.3 on the left. The discs were abnormally pale but had sharp edges and no protrusion. EEG had further improved but still showed a slow background activity. When last contacted for follow up in April 1969—6 years after her dramatic illness—she was still in the same good condition and had no difficulties in carrying out her school work.

**Comments.** This is a purely idiopathic case and a severe one. Of interest are the recurrent episodes of headache and vomiting occurring during the 3 years preceding her real illness. Histories of this type are not seldom found in the literature concerning purely idiopathic cases.

## ETIOLOGY

Being a diagnosis of exclusion several different explanations have been presented for pseudotumor cerebri and its etiology (Table 2).

One of the endocrine factors of main importance is undoubtedly a prolonged steroid therapy state which occurs in connection with the pseudotumor syndrome practically only in children (17-75). Any corticosteroid or ACTH preparation may be involved but triamcinolone has been reported surprisingly often (17). Many different diseases treated with steroids or ACTH and complicated by abnormally raised intracranial pressure have been reported (8-9, 14, 27, 33, 45, 73, 75). Children receiving long term steroids for asthma, rheumatoid arthritis or blood dyscrasias are characteristic victims (78). Symptoms of pseudotumor have most often occurred during rapid reduction or withdrawal of the corticosteroid regimen (33, 66).

Table 2 Different abnormal conditions associated with pseudotumor cerebri

- 1 Endocrine conditions
  - Hypoadrenism (14, 47, 75)
  - Hypothyroidism (59)
  - Hypoparathyroidism (1, 16, 71)
  - Pseudohypoparathyroidism (16)
  - Hyperadrenism (31)
  - Hyperthyroidism (29)
  - Ovarian dysfunction (34, 35, 51)
  - Oral contraceptive use (77)
  - Corticosteroid patients (14, 17, 30, 33, 66, 75)
- 2 Metabolic dysfunction
  - Vitamin A deficiency (2, 10)
  - Vitamin A overdosage (48, 61)
  - Vitamin B deficiency (44)
  - Uremia/pseudotumor (40)
  - Hypophosphatemia (25, 47)
  - Obesity (6, 36, 60)
- 3 Non-endocrine or non-metabolic systemic diseases
  - Anemias of different types (37, 40, 47, 50, 69)
  - Polycythemia vera (46)
  - Leukemia (21)
  - Aldrich's syndrome (37)
  - Chronic respiratory insufficiency (45)
  - Alkalosis (78)
  - Congenital or acquired heart disease (45, 81)
- 4 Allergic conditions
  - Food allergy (43)
  - Drug allergy (4, 45, 51, 68, 76)
  - Allergic diseases (15)
- 5 Non-cerebral infections
  - Otitis media (19, 30, 52, 72)
  - Paranasal sinusitis (19, 30, 52, 72)
  - Eukaryosis (19, 50, 52, 72)
  - Polymyositis (23, 54)
  - Roseola infantum (38)
  - Gonorrhea/Barré's syndrome (24)
- 6 Other conditions
  - Lymphoplastic encephalopathy (?)
  - Skull trauma (3, 6, 23)
- 7 Idiopathic states (37)

In adulthood one peculiarity is that 2/3-3/4 of the patients are women (6, 22, 74) particularly obese ones. An abnormal estrogen metabolism has therefore been implicated owing to the common findings of association with menstrual edema, premenstrual tension, menstrual irregularities, menarche, menopause, pregnancy, miscarriage or puerperal periods. However, no objective support from endocrinological studies has been obtained (57, 78). Such studies speak more in favour of a defect in the pituitary-corticotropin-adrenal axis (57).

The connection between pseudotumor cerebri and obesity is still more obscure but is



## Case 1

M A. This girl was born June 4 1951 as the first child in a healthy family. Her earlier years had been uneventful except for an alternating squint diagnosed when she was 3 years old. Because of headache when reading she was given glasses in 1962 and markedly improved.

During the autumn 1963 she developed 1-3 times per week a new form of headache particularly starting early in the morning. The headache was localized diffusely to both sides of the forehead and the parietal regions. Sometimes it was associated with nausea. After about one year a marked deterioration occurred. The headache appeared every morning and she felt dizzy and sick but never vomited. This deterioration coincided with her menarche which appeared August 1963 and during the following months her symptoms were exaggerated during the menstrual period.

In October 1964 when 13 1/2 years old she was examined at the pediatric department in the county hospital. She was found to be large and well developed for her age. General and neurological examination showed nothing abnormal except for the eye findings. She still had an alternating squint. Her vision was 0.3 on the right side 0.9 on the left. Both discs were edematous and protruding about 3 D. Skull X-ray was normal. EEG showed slight suppression of the basal activity but no focal abnormalities. Otolaryngological findings were normal.

The girl was sent to the University Hospital of Uppsala with a suspected intracranial tumor. Neuro-radiological examinations were planned but were postponed owing to gradual improvement. About 1 month later her vision was found to be 1.0 bilaterally and the disc protrusion had diminished. In December 1964 the discs were quite normal.

When last seen in July 1968 at her 5 year follow-up examination this girl still complained of headache during certain periods but was otherwise healthy. Her vision was found to be 0.9 bilaterally and the ocular fundi were normal. Nothing abnormal was found on neurological examination.

*Comments:* This teenage girl is one among the known cases where a pseudotumorous state appears in a girl during puberty and with a seemingly close connection between exaggeration of the symptoms and her menstrual period. It would seem highly probable that endocrine factors might have been of importance for the development of the syndrome even if the mechanism remains quite obscure.

## Case 2

R B. A boy 4 1/2 years old born February 3 1963 previously healthy 4th child in a healthy family. He was treated at the ENT department, University Hospital of Uppsala, October 9-13 1967 with penicillin for a left sided peritonsillitis but was sent home afebrile and in a good condition. One day later he became tired and apathetic and slept almost the whole day. During the next few days he vomited several

times gradually started to squint and developed a slight ptosis on the right side.

When examined at the pediatric department on October 17 1967 he shifted between a state of apathy and one of hyperirritability. He was slightly ataxic and could not walk without support. He had a left Vth nerve palsy protruding discs (2-3 D) and peripapillary streaks of hemorrhage. REG was diffusely abnormal without any normal background activity and showed irregular delta activity particularly on the right occipital region. X-ray revealed widened sutures. Echo-encephalography gave normal findings with an ordinary midline echo. An intracranial abscess was suspected and he was put on high doses of penicillin.

The boy was transferred to the neurosurgical department where lumbar encephalography and ventriculography were performed. These examinations revealed nothing abnormal except for marked difficulties in getting air to the convexity of the brain well consistent with a brain edema.

During the next few weeks the boy gradually improved but one month after appearance of the first symptoms he still had a disc protrusion of 2 D a left sided Vth nerve palsy and slight ataxia. During the next year his ataxia gradually disappeared but the other signs remained unchanged.

When last seen in July 1969 he was in a very good general condition, neurologically normal and now without any sign of Vth nerve palsy. His discs were still blurred but only slightly protruding and his vision was quite normal.

*Comments:* This 4 1/2 year-old boy gives an example of a pseudotumorous state probably due to parasitic infectious mechanisms.

## Case 3

A M. This girl born October 6 1956 was the third of four children in a healthy family. Her earlier psychomotor development had been quite normal and she passed her first school year with good marks. During the years 1960-63 she had periods of headache and vomiting lasting 1-2 days with free intervals of some months. She was examined by a pediatrician in April 1963 but nothing abnormal was revealed.

On the 28th of July 1963 when 7 1/2 years old she again had an acute attack of headache mainly right frontal and in addition vomiting lasting about 24 hours. After two days she became dizzy but had double vision and developed an unsteady gait. Because of increasing apathy and tiredness she was sent to the pediatric department of the county hospital. On examination she was found to have bilateral Vth nerve and right Vth nerve palsy, nystagmus, no corneal reflex on the left side and papilloedema with a disc protrusion of 2-3 D bilaterally. She was sent to the University Hospital of Uppsala on the 4th of August suspected of having a brain tumor. Air encephalography since burr holes had been made revealed a normal ventricular system with no displacement. The CSF was normal. EEG was severely ab-

normal with no normal background activity and diffuse symmetrical paroxysmal episodes. During the next month she deteriorated in spite of steroid treatment with 8 mg Dexamethasone  $\frac{1}{2}$  per day.

Because of increasing pain rapidly decreasing visual acuity nearly all blindfolded and protrusions of 1 D and abundant leakages of CSF through the berr holes bilateral subtemporal decompression was performed in September. Her general condition and vision thereafter improved. One month later her vision was 0.2 on the right eye and finger counts on the left. During the following month further improvement in vision and EEG were recorded.

In 1965 two years after the onset of her disease she had no headache and had passed her 4th school year without difficulties. Her temperament was said to be more labile than before the disease. Her VIIth and VIIIth nerve pulses had completely disappeared. The vision was 0.7 on the right eye and 0.3 on the left. The discs were abnormally pale but had sharp edges and no protrusion. EEG had further improved but still showed a slow background activity. When last contacted for follow up in April 1969—6 years after her dramatic illness—she was still in the same good condition and had no difficulties in carrying out her school work.

**Comments:** This is a purely idiopathic case and a severe one. Of interest are the recurrent episodes of headache and vomiting occurring during the 3 years preceding her real illness. Histories of this type are not seldom found in the literature concerning purely idiopathic cases.

## ETIOLOGY

Being a diagnosis of exclusion several different explanations have been presented for pseudotumor cerebri and its etiology (Table 2).

One of the endocrine factors of main importance is undoubtedly a prolonged steroid therapy state which occurs in connection with the pseudotumor syndrome practically only in children (17, 75). Any corticosteroid or ACTH preparation may be involved but triamcinolone has been reported surprisingly often (17). Many different diseases treated with steroids or ACTH and complicated by abnormally raised intracranial pressure have been reported (8, 9, 14, 27, 33, 45, 73, 75). Children receiving long term steroids for asthma, rheumatoid arthritis or blood dyscrasias are characteristic victims (78). Symptoms of pseudotumor have most often occurred during rapid reduction or withdrawal of the corticosteroid regimen (33, 66).

Table 2 Different abnormal conditions associated with pseudotumor cerebri

- 1 Endocrine conditions
  - Hypoadrenalism (14, 42, 75)
  - Hypothyroidism (39)
  - Hypoparathyroidism (1, 16, 71)
  - Pseudohypoparathyroidism (16)
  - Hyperadrenalism (31)
  - Hyperthyroidism (29)
  - Ovarian dysfunction (34, 35, 51)
  - Oral contraceptive use (77)
  - Corticosteroid patients (14, 17, 30, 33, 66, 75)
- 2 Metabolic dysfunction
  - Vitamin A deficiency (2, 10)
  - Vitamin A overdosage (48, 61)
  - Vitamin B deficiency (44)
  - Uremia/pseudoencephalopathy (44)
  - Hypophosphatemia (25, 43)
  - Obesity (6, 36, 60)
- 3 Non-endocrine or non-metabolic systemic diseases
  - Anemia of different types (37, 40, 47, 65, 69)
  - Polycythemia vera (46)
  - Leukemia (21)
  - Aldrich's syndrome (37)
  - Chronic respiratory insufficiency (45)
  - Alkalosis (76)
  - Congenital or acquired heart disease (45, 81)
- 4 Allergic conditions
  - Food allergy (45)
  - Drug allergy (4, 43, 68, 76)
  - Allergic diseases (15)
- 5 Non-cerebral infections
  - Otus media (19, 50, 52, 72)
  - Peritonsillar abscess (19, 50, 52, 72)
  - Ethmoiditis (19, 50, 52, 72)
  - Poliomyelitis (28, 54)
  - Roseola infantum (58)
  - Graftman-Barré's syndrome (24)
- 6 Other conditions
  - Lymphogenous encephalopathy (26)
  - Skull trauma (3, 6, 23)
- 7 Idiopathic states (18)

In adulthood one peculiarity is that 2/3-3/4 of the patients are women (6, 22, 74) particularly obese ones. An abnormal estrogen metabolism has therefore been implicated owing to the common findings of association with menstrual edema, premenstrual tension, menstrual irregularities, menarche, menopause, pregnancy, miscarriage or puerperal periods. However, no objective support from endocrinological studies has been obtained (57, 78). Such studies speak more in favour of a defect in the pituitary-corticotropin-adrenal axis (57). The connection between pseudotumor cerebri and obesity is still more obscure but is

repeatedly reported (12, 22 36 41 60) A rapid gain in weight was closely related to the onset of the raised intracranial pressure in six patients reported by Bradshaw (6)

Vitamin deficiency or overdosage states have occurred mainly in infants and often in connection with other notable symptoms and signs Thus, deficiency of vitamin A (2, 10) and vitamin B in pellagra (44) have been present in pseudotumor cases The same also applies to acute (48) and chronic (61) vitamin A intoxication, the latter being of particular diagnostic interest in infancy where the characteristic symptomatology of older children is lacking

Anemia due to iron deficiency (40 47) vitamin B<sub>12</sub> deficiency (65) hemolytic conditions (37) or blood loss (69) has been suggested as an etiologic factor but even true polycythemia has been considered to be the cause (46) The same also applies to other hematological disorders, among others leukemic conditions (21) and Aldrich's syndrome (37)

Many states leading to chronic hypoxia e.g. chronic respiratory insufficiency congenital (45) or acquired (81) heart disease, have been blamed as a cause of pseudotumor

Allergy in its various forms has been regarded as a possible contributory factor in the etiology (15) but its suggested mechanisms have permitted no critical evaluation (45) Recently a patient with a delayed penicillin reaction considered to be due to increased intracranial pressure was presented (68) The authors suggested this etiology because of the close association with a serum sickness like reaction, the marked worsening of the patient's condition following a benzathine procaine penicillin G injection and the strikingly positive reaction to a penicilloyl polylysine skin test

Tetracyclines (20 56) and nalidixic acid (4 53 76) are the most common drugs associated with benign intracranial hypertension but their possible allergic background has not yet been proved

Previous infections (excluding the acute stages of encephalitis and meningitis) have of

Table 3 Probable etiology in 18 cases of pseudotumor cerebri

	Infants	Children	Total
Paramfectious state (upper resp tract)	1	3	4
Reduction of steroid doses	3		3
Ovarian dysfunction in menarche		2	2
Obesity of a marked degree		2	2
Skull trauma Posttraumatic state (?)		1	1
Tetracycline	1		1
Anemia (autoimmune hemolytic)		1	1
Unknown		4	4
	5	13	18

ten been said to be responsible for increased intracranial pressure in children The most important are otitis media and paranasal sinusitis (19 52 72) but other infections in the upper respiratory tract and elsewhere in the body may also be the cause (13 22) Pseudotumorous states usually develop days or weeks after the acute stage usually after a free interval or in the later convalescent stage as in poliomyelitis (32 54)

A minimal to moderate skull trauma with or without a short-term loss of consciousness (66) or even with skull fracture (6) has given rise to a clinical picture of pseudotumor Because the head injury may have been rather trivial and the free interval between the injury and clinical symptoms and signs may vary from some days to 18 months (3) it may be difficult to associate these two conditions causatively with confidence

Finally there are also some cases often of older children, with no apparent clinical association so called purely idiopathic cases Such cases comprised 44 of the pediatric series of Rose & Matson (66) and are even more common in older ages (38)

In our material, a simple infection of the upper respiratory tract including the ears was considered to be the most common etiology (Table 3) which is well in agreement with the pediatric series of cases reported by Rose & Matson (66) A history of reduced dosage of

Table 4 Suggested pathogenetic mechanisms in pseudotumorcerebri

- I Cerebral edema
  - 1 Hypoxia
  - 2 Electrolyte imbalance
  - 3 Allergy
  - 4 Toxic mechanisms
  - 5 Pituitary ACTH adrenal axis insufficiency
  - 6 Other endocrine mechanisms
- II Interference with absorption of CSF
  - 1 Obstruction of dural venous outflow
  - 2 Spinal protein blockage
  - 3 Obstruction of cerebral lymph flow

steroids was obtained in three infants. All of these three patients had a pure red cell anemia but the hemoglobin was no longer at the bottom level when the onset of brain symptoms occurred. Ovarian dysfunction associated with menorrhoeal troubles was regarded as probably responsible in two cases. Obesity of a marked degree was present in connection with a pseudotumorous state in one boy 19 years of age who weighed 110 kg and had pronounced striae all over the body. The patient was suspected of having a pituitary tumor but extensive neuroradiological and neuroendocrinological investigations with a follow up of 1½ years demonstrated no evidence of a tumor. Another obese boy was also found to have normal 17 keto-steroids and 17 hydroxysteroids in the urine.

### PATHOGENESIS

Cerebral edema has most often been suggested to be the common factor of the pseudotumor our state (Table 4). The edema is probably subtle (78) and different (66) from the usual type which is present, among other conditions in acute toxic encephalopathies (18) and is accompanied by a typical lowered level of consciousness not characterizing pseudotumor (6, 13, 66, 80). Goebell (30) has tabulated 6 different supporting points that speak for cerebral edema as the pathogenetic mechanism in this condition. As the prognosis for complete recovery in pseudotumor is good (66) only oc-

casional autopsies have been reported (5, 9, 42). They have consistently revealed cerebral edema as the main feature. In addition Sahs & Joynt (67) in their biopsy material from 10 patients in decompressing operations have demonstrated both extracellular and intracellular edema in brain tissue.

Cerebral edema may develop in many ways. The blood brain barrier and the membrane function of the brain cells may be damaged by hypoxia in anemias of different types in congenital and acquired heart disease in chronic pulmonary insufficiency and in polio-myelitis with respiratory palsy. Electrolyte imbalance has been considered responsible in hypocalcemic tetany in hypoparathyroidism where differences in calcium concentrations extra- and intracellularly might cause accumulation of fluid in the cells. Steroid and ACTH therapy and hyperadrenalism may in some cases lead to hyponatremia and "water intoxication" (17). The blood brain barrier may also be damaged by toxins from infectious processes inside or outside the skull (22, 50). Various drugs may act in the same way (4, 53, 68, 76).

Suppression of adrenal cortical activity has been considered by many authors to be a pathogenetic mechanism of pseudotumor. Thus Jefferson (42) and Dees & McKay Jr (14) regarded it as a probable mechanism in Addison's disease and Walker & Adamkiewicz (75) in long term steroid therapy states on the basis of their reviewed 24 cases. Evidence of adrenal hypofunction was revealed by low 17-ketosteroids and 17 hydroxy ketosteroids in urine (14, 75).

Later Oldstone (57) in six female patients with pseudotumor found normal levels of female sex hormones, 17 ketosteroids and 17 hydroxy-corticosteroids in urine but no response to metopirone. He then concluded that ovarian dysfunction per se plays no role in the pathogenesis which is instead a hypofunction of the pituitary ACTH adrenal axis due to failure in the synthesis or release of endogenous ACTH. Whatever the mechanism the result is

a brain edema (60-75) which has been demonstrated at autopsy in cases of Addison's disease (42).

Dural sinus thrombosis is not considered to be guilty of interference with CSF absorption but to promote cerebral swelling (49-63-64-72-74). The causal relationship of superior longitudinal sinus thrombosis and pseudotumor—which is a common combination—can be most easily explained (74) namely by obstruction of bilateral venous outflow in the confluens sinuum. There must be additional factors causing a bilateral obstruction when lateral sinus thrombosis is instead present. These factors probably imply an interference with the by-pass of CSF (79).

### CLINICAL FEATURES IN INFANCY AND CHILDHOOD

Pseudotumor cerebri is an unusual condition in childhood. Davidoff (12) reported 12 patients below 12 years of age among 81 cases and in a recent report from Guidetti *et al* (38) there were only 7 children 0-10 years of age among their 100 cases. Purely pediatric series have been published among other authors by Eckler *et al* (17) and Rose & Matson (66).

The symptoms and signs (Table 5) are very similar from case to case and usually only differ in degree of severity. There is however reason to differentiate between the clinical picture in infants and that in older children.

The clinical picture in infancy is much more meager in symptoms and signs and more acute in its onset than in later years. Characteristically a simple upper respiratory infection with or without antibiotic therapy has preceded the onset by a few days or a week. A tense and bulging fontanel may be the only sign but not seldom in our experience the infant is also irritable, listless and dull. This is in contrast to the opinion of Bradshaw (6) who was impressed by the surprising alertness of the patients in association with their increased intracranial pressure. Vomiting and feeding difficulties are common. The head is usually not noticeably

Table 5 Clinical symptoms and signs in pseudotumor cerebri

1	General symptoms
	Headache
	Nausea and vomiting
	Dizziness
	Tinnitus
2	Signs localized to the skull
	Bulging fontanel
	Suture diastasis
	Cracked pot sound
	} in infants
3	Ophthalmological signs
	Papilledema, dilated veins
	Protruding discs
	Blurring of vision
	Visual hallucinations
	Diplopia
	Strabismus
	Visual field defects
	Nystagmus
4	Balance abnormalities
	Gait disturbance
	Other ataxic signs
5	Mental signs
	Apathy, lethargy (seldom)
	Irritability
	Nervousness
	Disturbed memory
6	Convulsive signs
	General convulsions
	Focal convulsions (rare)
7	Other neurological signs
	VIIth nerve palsy
	VIIIth nerve weakness
	Facial numbness
	Pyramidal signs
	Speech defects

enlarged but widened sutures are regularly found within some days or weeks. A cracked pot sound may be heard on percussion of the skull. Papilledema may or may not be present.

Older children complain of intermittent headache of usually moderate or even mild intensity. The headache is often localized to the frontal regions. Characteristically in the idiopathic cases there have often been episodes of less intense but the same sort of aching even months or years before the eventual and more multifarious picture has developed. Nausea, vomiting, dizziness and tinnitus may be added in various degrees. Visual impairment often appears early, distinguishing the condition from genuine cerebral tumor. When the first

symptoms of visual impairment appear a diagnosis of bilateral papilloedema has nearly been made. In severe cases it is connected with dilated veins of the ocular fundi, hemorrhages and papillary protrusion up to 9 D (30). Weakened visual power may be the cause of nystagmus. Diplopia and squint due to unilateral or more commonly bilateral VIth nerve palsy in addition to visual field defects make the patient feel unstable. Gait disturbances result secondarily and these may be further increased by cerebellar ataxia and pyramidal disorders due to the brain lesion. Focal neurological signs are only met exceptionally. In only a few cases have facial weakness or numbness or speech defects been noted.

Irritability, nervousness or apathy may be observed also in older children. Disorientation and disturbed memory is found in severe cases. General convulsions may occur in 10-20% of older patients and more frequently in children (17). Focal fits are rare. Acute states of increased blood pressure have also been described.

Even in the presence of severe neurological symptoms and signs the general condition and the intellectual capacity in many patients remain unimpaired.

A summary of complaints and clinical findings in our own material is shown in Table 1. Every patient with closed cranial sutures had protruded discs or at least unsharp disc edges and dilated veins in the ocular fundi. In the other cases all infants bulging fontanelles and suture diastases were regularly found.

### SPECIAL CLINICAL EXAMINATIONS

Electroencephalographic examination shows normal findings in a high percentage of the cases (30-70%) or reveals generalized changes with slow suppressed background activity and bilateral paroxysmal bursts (66) but no specific changes and no focal abnormalities (3).

Findings of normal constituents of the CSF are the rule. A protein level of higher than

50 mg% was only seen in 2 of 42 cases reported by Bradshaw (6). The CSF pressure is generally raised but is often considered to be lower than might be expected from the degree of disc protrusion in the ocular fundi. Thus Guzzetti *et al.* (38) measured the pressure at lumbar level in 76 cases and found it normal in 39, moderately raised in 21 and more so only in 16. Surprising and marked fluctuations in CSF pressure from day to day were recorded by Bradshaw (6).

Air encephalography should according to definition give normal or near normal findings. This was also the case in 69 of 100 patients in the recent series of Guzzetti *et al.* (38). These authors found small ventricles in 20 cases and slight symmetrical enlargements in 11. The same authors also strongly plead for the use of carotid angiography for diagnostic support. They refer to a special angiographic pattern including a characteristic stretching and widening of the curve of the anterior cerebral artery in lateral views in the simultaneous absence of pneumographic evidence of expanding hydrocephalus. This is in contrast to the findings of Goebell (30) who performed 42 carotid angiographies of a serial type illustrating all stages of cerebral circulation and assessed their 42 cases as normal.

### DIAGNOSIS

The most important point for an early diagnosis is awareness of the existence of this peculiar syndrome. The diagnosis is then based on exclusion of localized expansive processes and on the history, clinical findings and—if present—demonstration of hypertension of the CSF at lumbar or ventricular puncture. Other supporting signs may be the demonstration of dural sinus thrombosis in cerebral angiography and signs of raised intracranial pressure in the plain skull X-ray. A normal or small ventricular size and a non-displaced ventricular system is essential. EEG usually gives no support for the diagnosis.

## DIFFERENTIAL DIAGNOSIS

*Pseudotumor cerebri* is mainly a diagnosis of exclusion. Therefore the importance lies on exhaustive studies to exclude all possible expansive or acute infectious intracranial processes in the cerebrum and the meninges (Table 6). *Hemispherical tumors* are usually diagnosed without difficulties: cortical ones give rise to focal symptoms and signs at clinical examination, focal changes in EEG and positive findings at carotid angiography. Subcortical medial hemisphere tumors are best discovered by air encephalography. Usually they deform and displace the ventricular system in addition to giving localizing signs, but they may provide difficulties when small and slowly growing. Aneurysms are demonstrated by angiography, as well as hemorrhage which in addition commonly leads to loss of consciousness and bloody CSF.

In infancy *subdural effusions* may give very similar clinical symptoms and signs. Subdural taps and carotid angiography give a differentiation. Echo encephalography may be of value in certain cases of subdural processes with abnormal lateral echoes. When papilledema is present subdural effusions are characteristically often combined with peripapillary hemorrhages.

*Expansive hydrocephalus* is readily diagnosed by demonstrating signs of raised intracranial pressure associated with markedly dilated ventricles.

*Encephalitis*, *meningitis* and *lead poisoning* usually give increased cells and/or proteins in CSF. The clinical findings otherwise cannot be sharply delimited from those in benign intracranial hypertension. *Vascular hypertension* can readily be excluded but one must remember that vascular hypertension may on the other hand occur secondarily to increased intracranial pressure also in cases of pseudotumor. Bradycardia is then mostly associated.

Particularly in generalized *craniostenosis* the brain is secondarily compressed giving rise to strikingly bulging fontanelles and/or papilledema, but the premature closure of sutures

Table 6 Conditions of importance for differential diagnosis of pseudotumor cerebri

- 1 Cerebral tumor  
Neoplasms  
Cysts aneurysms  
Hemorrhages  
Abscesses
- 2 Subdural processes  
Subdural hemorrhage  
Subdural effusions
- 3 Expansive hydrocephalus  
Obstructive forms  
Overproductive forms
- 4 Encephalitis
- 5 Meningitis
- 6 Lead poisoning
- 7 Vascular hypertension
- 8 Optic neuritis (or atrophy?)
- 9 Pseudo-papilledema in  
Hyperopia  
Thrombosis of central vein  
Deformed optic discs
- 10 Craniostenosis
- 11 Constitutional pseudopapillitis

is readily diagnosed both clinically and with plain skull X rays.

Isolated optic disc changes, especially *optic neuritis* may be of infectious or degenerative origin and may sometimes be diagnosed erroneously as pseudotumor with papilledema (11). Pathologic changes in CSF, the early and severe impairment of vision and a failure to show a raised CSF pressure will together contribute towards a correct diagnosis. Also other anomalies of the optic discs or eyeballs may mistakenly give rise to a suspicion of papilledema (28-31) but such conditions are usually isolated occasional findings and remain unchanged in follow up studies.

## TREATMENT

*Pseudotumor cerebri* is usually a benign condition which needs no therapy. However careful observation is always needed, especially in fulminant stages. Diuretics, e.g. acetazolamide (37) or furosemide (66) or restriction of fluid and salt only seldom have any effect. Pseudotumor induced by withdrawal of steroid or

ACTH is treated by a short term increase of the doses of steroids. Endocrine correcting therapy is of course the treatment of choice in hormonal hypo- or hyperfunctional conditions as well as adequate substitution in anaemia and metabolic deficiency states.

Operative measures are mainly indicated when visual function is threatened. According to Holmes (39) loss of vision may be particularly suspected in the following situations (if primary eye disease has been excluded): 1) rapidly developing papilledema, 2) high grade papilledema (over 5 D) with retinal hemorrhages and exudates, 3) marked retinal arteriolar constriction, 4) narrowing of the visual fields and 5) brief periodic black-outs even if papilledema is not severe. Repeated lumbar punctures can only temporarily correct such conditions (6). Therefore subtemporal decompression or nowadays preferably shunt operations are highly recommended in impending visual loss. Excision of occluding sinus thrombi can be performed in selected cases.

## COURSE AND DURATION OF ILLNESS

The duration of the pseudotumorous state may vary from some days up to some few years. However, most of the cases are free from papilledema 6 months after the onset (66). Other symptoms and signs of pseudotumor have a still shorter duration. Recurrences are uncommon particularly in childhood. Permanent impairment of vision may remain in severe cases but the outcome in general is considered in the literature to be good or even excellent (13, 37). The most recent follow up study was made by Guxley *et al.* (38). All of his 80 patients had become free from neurological symptoms and signs. Twelve cases had a residual visual defect. We had the same good experiences from our series. No deaths were recorded. A definitely impaired vision was only noted in the most severe of our cases (case 3) and three others had some slight residual neurological abnormalities. All the rest had completely recovered.

## SUMMARY

Clinical experiences from 118 infants and children with a diagnosis of pseudotumor cerebri and cared for in Uppsala and Turku during the years 1960-69 have been summarized. Three case reports with clinical pictures of particular interest have been given in more detail. In addition a general review of this topic has been given.

## ACKNOWLEDGEMENTS

This investigation was supported by grants from Otho and Elin Eriksson's foundation for scientific research and the Swedish medical research council projects no B68 19X 108-05A and K69 95F 2762-01.

## REFERENCES

1. Barr D P, McBryde C M & Sanders T E. Tetany with increased intracranial pressure and papilledema. *Trans Am Amer Physicians* 53: 217 1938.
2. Bass M H & Caplan J. Vitamin A deficiency in infancy. *J Pediatr* 47: 690 1955.
3. Beller A J. Benign post-traumatic intracranial hypertension. *J Neurol Neurosurg Psychiatr* 27: 149 1964.
4. Boren L O & Sandstrom B. Intracranial hypertension in a child during treatment with salicylic acid. *Brit Med J* II 744 1967.
5. Boudin G, Funch-Brentano J L & Gayno M. Considerations sur une ocephalopathie addisonienne. *Bull Soc Med Hop Paris* 66: 1741 1950.
6. Bradshaw P. Benign intracranial hypertension. *J Neurol Neurosurg Psychiatr* 19: 28 1956.
7. Buchheit W A, Burton C, Haig B & Shaw D. Papilledema and idiopathic intracranial hypertension: report of a familial occurrence. *Am Eng J Med* 280: 938 1969.
8. Calzavara P L & Rubin M I. Physiologic considerations concerning corticosteroid therapy and enough studies in the nephrotic syndrome. *J Pediatr* 58: 686 1961.
9. Chaput J, Jean R & Bonnet, H. Syndrôme pseudotumoral mortel par déprivation surrénale. Tableau d'hypertension cérébrale fatale au cours d'une néphrose lipidique avec arrêt brusque du traitement corticoïdique. *Acta Neuropath* 10: 45 1968.
10. Cornfield El & Cooke R E. Vitamin A deficiency case report. Unusual manifestations in a 3 1/2 month old baby. *Pediatrics* 10: 33 1952.
11. Dandy W E. Intracranial pressure without brain tumor. *Ann Surg* 106: 492 1937.
12. Davidoff L M. Pseudotumor cerebri. Benign intracranial hypertension. *Neurology* 6: 605 1956.



- 13 Davidoff L M & Dyke C G A presentation of a series of cases of serous meningitis *J Nerv Ment Dis* 83 700 1936
- 14 Dees S C & McKay H W Jr Occurrence of pseudotumor cerebri (benign intracranial hypertension) during treatment of children with asthma by adrenal steroids: Report of three cases *Pediatrics* 23 1143 1959
- 15 Devenney J W Jr & Shea J H Allergic encephalopathy with papilledema *Arch Neurol Psychiat* 68 791 1952
- 16 DiGeorge A M Hypoparathyroidism In W E Nelson (ed) *Textbook of Pediatrics* W B Saunders Company Philadelphia 1964 p 1284
- 17 Eckler E Schonberg D & Brench J R Pseudotumor cerebri infolge Corticosteroidtherapie *Misch Kinderheilk* 114 271 1966
- 18 Eiben R M Acute brain swelling (toxic encephalopathy) *Pediat Clin N America* 14 797 1967
- 19 Erner M S & Myers D Otic hydrocephalus with a suggestion as to etiology *Ann Otol* 45 553 1936
- 20 Fields J P Bulging fontanel: a complication of tetracycline therapy in infants *J Pediatr* 58 74 1961
- 21 Fleury D D P Verhoogde intracraniele druk als eerste symptoom bij een kind met acute leukemie *Nederl T Geneesk* 112 1170 1968
- 22 Foley J Benign forms of intracranial hypertension—Toxic and otic hydrocephalus *Brain* 78 1 1955
- 23 Ford F R & Murphy E L Increased intracranial pressure: A clinical analysis of the causes and characteristics of several types *Bull Hopkins Hosp* 64 369 1939
- 24 Ford F R & Walsh F B Guillain Barre syndrome (acute infective polyneuritis) with increased intracranial pressure and papilledema *Bull Hopkins Hosp* 73 391 1943
- 25 Fraser D Yendt E R & Christie F H E Metabolic abnormalities in hypophosphatasia *Lancet* 1 286 1955
- 26 Foldi M Obál F Kahan A Wagner A Casada E & Borcsok E Lymphogene Enterophalopathie *Acta Paediat Acad Sci Hung* 8 171 1967
- 27 Gausford W Some diagnostic difficulties *J Pediatr* 56 259 1960
- 28 Gess H H Papilledema and pseudotumor cerebri following poliomyelitis *Amer J Dis Child* 93 640 1957
- 29 Glowacki J Guazzi G C Alvisi C Gambetti P Jonckheer M & Tassinari C A Loedème cérébral pseudotumoral endocrinien et ou métabolique *Acta Neurol Belg* 65 873 1965
- 30 Goebell H Die Stauungspapille unklarer Genese Ein Beitrag zum Bild des Pseudotumor cerebri *Fortschr Neurol Psychiat* 32 265 1964
- 31 Gordon R C & Kelsey W M Pseudotumor cerebri in congenital adrenal hyperplasia *Amer J Dis Child* 113 727 1967
- 32 Granstrom A O Staspapill papilloedem opticus concurrent papillit pseudopapillit *Opuscula (Stockh)* 10 3 1965
- 33 Greer M Benign intracranial hypertension following corticosteroid therapy *Neurology* 13 439 1963
- 34 — Benign intracranial hypertension IV *Neurology* 14 569 1964
- 35 — Benign intracranial hypertension V Menstrual dysfunction *Neurology* 14 668 1964
- 36 — Benign intracranial hypertension VI Ocular *Neurology* 15 382 1965
- 37 — Benign intracranial hypertension (pseudotumor cerebri) *Pediat Clin N America* 14 1 1967
- 38 Guedetti H Giffire R & Gambacorta J Follow up study of 100 cases of pseudotumor cerebri *Acta Neurochir* 18 259 1968
- 39 Holmes G The prognosis in papilledema *Br J Ophthalm* 21 337 1937
- 40 Ikala E & Lantton L Papilloedema due to iron deficiency anaemia *Acta Haemat* 29 34 1963
- 41 Jacobson H G & Shapiro J H Pseudotumor cerebri *Radiology* 82 202 1964
- 42 Jefferson A A clinical correlation between intracranial cephalopathy and papilloedema in Addison's disease *J Neurol Neurosurg Psychiat* 19 21 1956
- 43 Jelle H Hypophosphatasia *Acta Paediat Scand* 49 297 1960
- 44 Kehrler F A Zur Frage des Pseudotumor cerebri *Deutsch Z Nervenheilk* 160 1 1949
- 45 Leck H I & Baker D Pseudotumor cerebri: an allergic phenomenon? A discussion of 17 cases including two of infants manifesting pseudotumor while receiving soybean seedlings *Clin Pediatr (Phila)* 4 32 1965
- 46 Loman J & Dameshek W Increased intracranial venous and cerebrospinal fluid pressures in polycythemia *Trans Amer Neurol Ass* 70 8 1944
- 47 Lubeck M J Papilledema caused by iron-deficiency anaemia *Trans Amer Acad Ophthalmol* 63 306 1959
- 48 Marie J & See J Acute hypervitaminosis A in the infant Its clinical manifestation with benign acute hydrocephalus and pronounced bulging of the fontanel: a clinical and biologic study *Amer J Dis Child* 87 731 1954
- 49 Martin J P & Sheehan H L Primary thrombosis of cerebral veins (following childbirth) *Brit Med J* 1 349 1941
- 50 McAlpine D Toxic hydrocephalus *Brain* 66 180 1937
- 51 McCullagh E P Menstrual edema with intracranial hypertension (pseudotumor cerebri) Report of a case *Cleveland Clin Quart* 8 707 1941
- 52 Millichap J G Benign intracranial hypertension and otic hydrocephalus *Pediatrics* 23 257 1959
- 53 Murahovsch J Scaff A de Andrade A J R Goldman L Racowski P & Crespin J Toxic

- mento das diarréias agudas da primeira infância com o ácido valilúrico *Pediatr Frib (S. Paulo)* 37 31 1966
- 54 Murray R G & Walsh P B Ocular motor palsies in polyomyelitis and their pathogenesis *Canad Med Ass J* 70 141 1954
  - 55 Noctor M Dor Pseudotumor cerebri *Neurochir Clin* 10 107 1914
  - 56 O'Doherty N J Acute benign intracranial hypertension in an infant receiving tetracycline *Develop Med Child Neurol* 7 677 1965
  - 57 O'Donoghue M B A The endocrinological aspects of benign intracranial hypertension *Arch Neurol* 15 362 1966
  - 58 Oski F A Rosolia subatum another cause of bulging fontanel *Amer J Dis Child* 101 376 1961
  - 59 Palmer R F Seales H H & Boldrey E B Papilledema and hypoparathyroidism simulating brain tumor *J Neurosurg* 16 378 1959
  - 60 Patterson R DePasquale N & Mann S Pseudotumor cerebri *Medicine* 40 85 1961
  - 61 Perren J Tunell R & Ekstrom K Chronic vitamin A intoxication during the first half year of life Description of 5 cases *Acta Paediatr Scand* 54 9 1965
  - 62 Quincke H Ueber serositas serosa *Savand Ann Fenn Soc Med* 23 655 1893
  - 63 Ray B S & Dober H B Thromboses of the superior sagittal sinus as a cause of pseudotumor cerebri methods of diagnosis and treatment *Trans Amer Neurol Ass* 75 12 1950
  - 64 — Thromboses of the dural venous sinuses as a cause of pseudotumor cerebri *Ann Surg* 134 376 1951
  - 65 Reid H A & Harris W Reversible papilloedema in pericardial anastomosis *Br Med J* 1 20 1951
  - 66 Rose A & Matson R D Benign intracranial hypertension in children *Pediatrics* 39 217 1967
  - 67 Sabin A L & Joynt R J Brain swelling of unknown cause *Neurology* 6 791 1956
  - 68 Schenck B D & Kruse W Benign intracranial hypertension associated with a delayed penicillin reaction *Pediatrics* 43 50 1969
  - 69 Schreiber J R & Blomberg A G Papilledema associated with blood loss anastomosis *Ann Intern Med* 55 1004 1961
  - 70 Silverman D Papilledema and the electroencephalogram *Neurology* 14 899 1964
  - 71 Stephen A Albright F & McCune D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moon faces *J Clin Endocr* 5 625 1943
  - 72 Symonds C P Ocular hydrocephalus *Brain* 54 55 1931
  - 73 Valentine G H Truncosclerosis and intracranial hypertension side effect? *Lancet* 1 892 1959
  - 74 Wagener H P Pseudotumor cerebri *Amer J Med Sci* 227 214 1954
  - 75 Walker A E & Adamkiewicz J J Pseudotumor cerebri associated with prolonged corticosteroid therapy Reports of four cases *JAMA* 184 779 1964
  - 76 Walker S H Salama I & Stansford W E Malabsorption and in childhood urinary infection clinical and laboratory experience with fifty patients *Clin Pediatr* 5 718 1966
  - 77 Walsh P B Clark H B Thompson R S & Nacholson D H Oral contraceptives and heterophthalmologic anisotropia *Arch Ophthalmol* 74 628 1965
  - 78 Wilson C B & Bertan V A review of factors in the pathogenesis of pseudotumor cerebri *Park J Pediatr* 9 125 1967
  - 79 Woodhall E Variations of cranial sutures in region of torcular herophili *Arch Surg* 33 297 1936
  - 80 Yankin J C Groff E A & Shook H A Severe papilledema in dilute solutions of the nervous system *Trans Amer Neurol Ass* 72 21 1947
  - 81 Zandbergen G D & Cohen S J Pseudotumor cerebri *J Neurosurg* 11 435 1954
- Submitted Sept 27 1969  
Accepted Nov 1 1969
- (B H) Dept of Pediatrics  
Akademiska sjukhuset  
Uppsala  
Sweden
- Key words** Benign intracranial hypertension pseudo tumor cerebri intracranial pressure papilledema

- 13 Davidoff L M & Dyke C B A presentation of a series of cases of serous meningitis *J Nerv Ment Dis* 83 700 1936
- 14 Dees S C & McHay H W Jr Occurrence of pseudotumor cerebri (benign intracranial hypertension) during treatment of children with asthma by adrenal steroids. Report of three cases *Pediatr* 23 1143 1959
- 15 Devenney J W Jr & Shea J H Allergic encephalopathy with papilledema *Arch Neurol Psychiatr* 68 791 1952
- 16 DiGeorge A M Hypoparathyroidism In W E Nelson (ed) *Textbook of Pediatrics* W B Saunders Company Philadelphia 1964 p 1284
- 17 Eckler E Schonberg D & Breich J R Pseudotumor cerebri infolge Corticosteroidtherapie *Machr Kinderheilk* 114 271 1966
- 18 Eiben R M Acute brain swelling (toxic encephalopathy) *Pediatr Clin N America* 14 797 1967
- 19 Eraser M S & Myers D Otic hydrocephalus with a suggestion as to etiology *Ann Otol* 45 533 1936
- 20 Fields J P Bulging fontanel a complication of tetracycline therapy in infants *J Pediatr* 59 74 1961
- 21 Fleury D D P Verhoogde intracraniele druk als eerste symptoom bij een kind met acute leukemia *Nederl T Geneesk* 112 1170 1968
- 22 Foley J Benign forms of intracranial hypertension—toxic and otic—hydrocephalus *Brain* 78 1 1955
- 23 Ford F R & Murphy E L Increased intracranial pressure. A clinical analysis of the causes and characteristics of several types *Bull Hopkins Hosp* 64 369 1939
- 24 Ford F R & Walsh F B Guillain Barré syndrome (acute infective polyneuritis) with increased intracranial pressure and papilledema *Bull Hopkins Hosp* 73 391 1943
- 25 Fraser D Yendi E R & Christie F H B Metabolic abnormalities in hypophosphataemia *Lancet* 1 286 1955
- 26 Foldi M Obdi F Kahin A Wagner A Carada E & Borzok E Lymphogene Enzephalopathie *Acta Laedist Acad Sci Hung* 8 171 1967
- 27 Gaisford W Some diagnostic difficulties *J Pediatr* 56 259 1960
- 28 Gass H H Papilledema and pseudotumor cerebri following poliomyelitis *Amer J Dis Child* 93 640 1957
- 29 Glowacki J Guzzi G C Alvim C Grubbe P Jonckheer M & Tashman C A Loedème cérébral pseudotumoral endocrinien ou métabolique *Acta Neurol Belg* 65 873 1965
- 30 Goebell H Die Stauungspapille unklarer Genese Ein Beitrag zum Bild des Pseudotumor cerebri *Fortschr Neurol Psychiatr* 37 263 1964
- 31 Gordon R C & Kelsey W M Pseudotumor cerebri in congenital adrenal hyperplasia *Amer J Dis Child* 113 727 1967
- 32 Granstrom A O Stauungspapill papilloedem opticoeunt papillit pseudopapillit *Opus Med* (Stockh) 10 3 1965
- 33 Greer M Benign intracranial hypertension II Following corticosteroid therapy *Neurology* 13 439 1963
- 34 — Benign intracranial hypertension IV *Menarche Neurology* 14 569 1964
- 35 — Benign intracranial hypertension V Menstrual dysfunction *Neurology* 14 668 1964
- 36 — Benign intracranial hypertension VI Obesity *Neurology* 15 382 1965
- 37 — Benign intracranial hypertension (pseudotumor cerebri) *Pediatr Clin N America* 14 819 1967
- 38 Guidetti B Gruffe R & Gambacorta D Follow up study of 100 cases of pseudotumor cerebri *Acta Neurochir* 18 259 1968
- 39 Holmes G The prognosis in papilledema *Brit J Ophthalmol* 21 337 1937
- 40 Ilkka E & Laitinen L Papilloedema due to iron deficiency anaemia *Acta Haemat* 79 368 1963
- 41 Jacobsson H G & Shapiro J H Pseudotumor cerebri *Radiology* 82 202 1964
- 42 Jefferson A A clinical correlation between encephalopathy and papilloedema in Addison's disease *J Neurol Neurosurg Psychiatr* 19 21 1956
- 43 Jelle H Hypophosphataemia *Acta Paediatr Scand* 49 297 1960
- 44 Kehrer F A Zur Frage des Pseudotumor cerebri *Deutsch Z Kinderheilk* 160 1 1949
- 45 Leck H I & Baker D Pseudotumor cerebri an allergic phenomenon? A discussion of 17 cases including two of infants manifesting pseudotumor while receiving soybean feedings *Can Pediatr (Phil)* 4 32 1965
- 46 Loman J & Dimeshek W Increased intracranial venous and cerebrospinal fluid pressures in polycythemia *Trans Amer Neurol Ass* 70 14 1944
- 47 Lubek M J Papilledema caused by iron-deficiency anaemia *Trans Amer Acad Ophthalm Otolaryng* 63 306 1959
- 48 Mäse J & See G Acute hypervitaminosis A of the infant Its clinical manifestation with benign acute hydrocephalus and pronounced bulge of the fontanel a clinical and biological study *Amer J Dis Child* 87 731 1954
- 49 Martin J P & Sheehan H L Primary thrombosis of cerebral veins (following childbirth) *Brit Med J* 1 349 1941
- 50 McAlpine D Toxic hydrocephalus *Brain* 60 180 1937
- 51 McCullagh E P Menstrual edema with intracranial hypertension (pseudotumor cerebri) Report of a case *Cleveland Clin Quart* 8 202 1941
- 52 Millachap J G Benign intracranial hypertension and otic hydrocephalus *Pediatrics* 23 257 1959
- 53 Murahovachi J Scaff A de Andrade A J R Goldman L Racowski P & Cresnan J Traita

- mento das diarrreias agudas da primeira infancia com o acido salicilico *Pediatr Bras* (S Paulo) 37 31 1966
- 4 Murray R G & Walsh P B Ocular abnormalities in polyarthritis and their pathogenesis *Canad Med Ass J* 70 141 1954
  - 5 Nause M Det Pseudotumor cerebri *Neue Druck Chr* 10 107 1914
  - 6 O'Doherty N J Acute benign intracranial hypertension in an infant receiving tetracycline *Develop Med Child Neurol* 7 677 1965
  - 7 Oldstone M B A The endocrinological aspects of benign intracranial hypertension *Arch Neurol* 15 362 1966
  - 8 Oski F A Roseola infantum another cause of bulging fontanel *Amer J Dis Child* 101 376 1961
  - 9 Palmer R F Seales H H & Boldrey E B Papilledema and hypoparathyroidism complicating brain tumor *J Neurosurg* 16 378 1959
  - 10 Peterson R DePasquale N & Mink S Pseudotumor cerebri *Med Clin* 40 85 1961
  - 11 Persson B Tazell R & Ekestigren K Chronic vitamin A intoxication during the first half year of life Descriptions of 5 cases *Acta Paediatr Scand* 54 49 1965
  - 12 Quacke H Ueber meningitis serosa *Saunders Atlas Vortr Les Med* 23 635 1893
  - 13 Ray B S & Dauter H S Thrombosis of the superior sagittal sinus as a cause of pseudotumor cerebri: methods of diagnosis and treatment *Trans Amer Neurol Ass* 75 12 1950
  - 14 — Thrombosis of the dural venous sinuses as a cause of pseudotumor cerebri *Ann Surg* 134 376 1951
  - 15 Reid H A & Harris W Reversible papill oedema in peritoneal scissures *Brit Med J* 1 20 1931
  - 16 Rowe A & Matson D E Benign intracranial hypertension in children *Pediatrics* 39 227 1967
  - 17 Saks A L & Joynt R J Brain swelling of unknown cause *Neurology* 6 791 1956
  - 18 Schmitt B D & Aron W Benign intracranial hypertension associated with a delayed penicillin reaction *Pediatrics* 43 50 1969
  - 19 Schwaber J R & Blumberg A G Papilloedema associated with blood loss anemia *Ann Intern Med* 55 1004 1961
  - 20 Silverman D Papilloedema and the electroen cephalogram *Neurology* 14 899 1964
  - 21 Solpian A Albright F & McCune D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moonface *J Clin Endocr* 3 625 1943
  - 22 Symonds C P Ocular hydrocephalus *Brain* 54 44 1931
  - 23 Valentine G H Trisacrinolone and intracranial hypertension side effect? *Lancet* 1 892 1959
  - 24 Wagener H P Pseudotumor cerebri *Amer J Med Sci* 227 214 1954
  - 25 Walker A H & Adamkiewicz J J Pseudotumor cerebri associated with prolonged corticosteroid therapy Reports of four cases *JAMA* 188 779 1964
  - 26 Walker S H, Salazar I & Standford W F Nephrotic acid in childhood urinary infection clinical and laboratory experience with fifty patients *Clin Pediatr* 5 118 1966
  - 27 Welsh F B Clark D B Thompson R S & Nicholson D H Oral contraceptives and neuro-ophthalmologic interest *Arch Ophthalmol* 74 628 1965
  - 28 Wilson C B & Berian V A review of factors in the pathogenesis of pseudotumor cerebri *Tex J Pediatr* 9 125 1967
  - 29 Woodhall II Variations of cranial sinuses in region of torcular herophili *Arch Surg* 33 297 1936
  - 30 Yaskan J C Groff R A & Shenkin H A Severe papilloedema in diffuse infections of the nervous system *Trans Amer Neurol Ass* 72 21 1947
  - 31 Zandema G H & Cohen S J Pseudotumor cerebri *J Neurosurg* 11 433 1954

Submitted Sept 27 1969

Accepted Nov 12 1969

(B H) Dept of Paediatrics  
Akademiska Sjukhuset  
Uppsala  
Sweden

Key words Benign intracranial hypertension pseudo tumor cerebri intracranial pressure papilloedema

- 13 Davidoff L M & Dyke C G A presentation of a series of cases of serous meningitis *J Nerv Ment Dis* 83 700 1936
- 14 Dees S C & McKay H W Jr Occurrence of pseudotumor cerebri (benign intracranial hypertension) during treatment of children with asthma by adrenal steroids Report of three cases *Pediatrics* 23 1143 1959
- 15 Devenney J W Jr & Shea J H Allergic encephalopathy with papilledema *Arch Neurol Psychiat* 68 791 1952
- 16 DiGeorge A M Hypoparathyroidism In W E Nelson (ed) *Textbook of Pediatrics* W B Saunders Company Philadelphia 1964 p 1284
- 17 Eckler E Schonberg D & Bianchi J R Pseudotumor cerebri infolge Corticosteroidtherapie *Mitschr Kinderheilk* 114 271 1966
- 18 Eiben R M Acute brain swelling (toxic encephalopathy) *Pediatr Clin N America* 14 797 1967
- 19 Erner M S & Myers D Otic hydrocephalus with a suggestion as to etiology *Ann Otol* 45 553 1936
- 20 Fields J P Bulging fontanel a complication of tetracycline therapy in infants *J Pediatr* 58 74 1961
- 21 Fleury D D P Verhoogde intracraniele druk als eerste symptoom bij een kind met acute leukemie *Nederl T Geneesk* 112 1170 1968
- 22 Foley J Benign forms of intracranial hypertension—toxic and otic hydrocephalus *Brain* 78 1 1955
- 23 Ford F R & Murphy E L Increased intracranial pressure A clinical analysis of the causes and characteristics of several types *Bull Hopkins Hosp* 64 369 1939
- 24 Ford F R & Walsh F B Guillain Barre syndrome (acute infective polyneuritis) with increased intracranial pressure and papilledema *Bull Hopkins Hosp* 73 391 1943
- 25 Fraser D Yeendt E R & Christie F H E Metabolic abnormalities in hypophosphatasia *Lancet* 1 286 1955
- 26 Foldi M Obál F Kahan A Wagner A Csanda E & Borcsák E Lymphogene Enzephalopathie *Acta Paediat Acad Sci Hung* 8 171 1967
- 27 Gausford W Some diagnostic difficulties *J Pediatr* 56 259 1960
- 28 Gass H H Papilledema and pseudotumor cerebri following poliomyelitis *Amer J Dis Child* 93 640 1957
- 29 Glowacki J Gurzu G C Alvaiz C Gambetti P Jonckheer M & Tassinari C A Loedeme cerebral pseudotumoral endocrinien et ou metabolique *Acta Neurol Belg* 65 873 1965
- 30 Goebell H Die Stauungspapille unklarer Genese Ein Beitrag zum Bild des Pseudotumor cerebri *Fortachr Neurol Psychiat* 32 265 1964
- 31 Gordon R C & Kelsey W M Pseudotumor cerebri in congenital adrenal hyperplasia *Amer J Dis Child* 113 727 1967
- 32 Granstrom K O Staspapill papillodem opticonjunct papillit pseudopapillit *Opac Med (Stockh)* 10 3 1965
- 33 Greer M Benign intracranial hypertension. II Following corticosteroid therapy *Neurology* 13 439 1963
- 34 — Benign intracranial hypertension IV *Neurology* 14 569 1964
- 35 — Benign intracranial hypertension V Menstrual dysfunction *Neurology* 14 668 1964
- 36 — Benign intracranial hypertension VI Ocular *Neurology* 15 382 1965
- 37 — Benign intracranial hypertension (pseudotumor cerebri) *Pediatr Clin N America* 14 819 1967
- 38 Gundetti B Giffre R & Gambacorta D Follow up study of 100 cases of pseudotumor cerebri *Acta Neurochir* 18 259 1968
- 39 Holmes G The prognosis in papilledema *Brit J Ophthalm* 21 337 1937
- 40 Ikkala E & Laitinen L Papilloedema due to iron deficiency anaemia *Acta Haemat* 29 389 1963
- 41 Jacobson H G & Shapiro J H Pseudotumor cerebri *Radiology* 82 207 1964
- 42 Jefferson A A clinical correlation between encephalopathy and papilloedema in Addison's disease *J Neurol Neurosurg Psychiat* 19 21 1956
- 43 Jelle H Hypophosphatasia *Acta Paediat Scand* 49 297 1960
- 44 Lehrer F A Zur Frage des Pseudotumor cerebri *Deutsch Z Kinderheilk* 160 1 1949
- 45 Leckis H I & Baker D Pseudotumor cerebri an allergic phenomenon? A discussion of 17 cases including two of infants manifesting pseudotumor while receiving soybean feedings *Chn Pediatr (Phila)* 4 32 1965
- 46 Loman J & Dameshek W Increased intracranial venous and cerebrospinal fluid pressures in polycythemia *Trans Amer Neurol Ass* 70 84 1944
- 47 Lubeck M J Papilledema caused by iron deficiency anaemia *Trans Amer Acad Ophthalm Otolaryng* 63 306 1959
- 48 Marie J & See G Acute hypertammosis A of the infant Its clinical manifestation with benign acute hydrocephalus and pronounced bulge of the fontanel a clinical and biologic study *Amer J Dis Child* 87 731 1954
- 49 Martin J P & Sheehan H L Primary thrombosis of cerebral veins (following childbirth) *Brit Med J* 1 349 1941
- 50 McAlpine D Toxic hydrocephalus *Brain* 60 180 1937
- 51 McCullagh E P Menstrual edema with intracranial hypertension (pseudotumor cerebri) Report of a case *Cleveland Clin Quart* 8 202 1941
- 52 Millichap J G Benign intracranial hypertension and otic hydrocephalus *Pediatrics* 23 257 1959
- 53 Murahovschi J Scaff A de Andrade J Goldman L Racowski P & Crespin J

x the chest showed no abnormality. A nasal swab was taken and she was started on a course of intracytins. Two days after the onset of the upper respiratory infection she suddenly collapsed and died.

Necropsy confirmed the diagnosis of osteogenesis imperfecta and death was considered to be due to the presence of a large subdural haematoma.

#### Case 2

R. Q. male one of twins was born in UCH on 31.1.67 at 36 weeks to a young primigravida. He is the second twin and weighed 1800 g at birth. The skull showed asymmetry with multiple defects and large anterior fontanelle. The lower legs were short and bowed and there was evidence of fractures of both tibiae and fibulae. The sclerae were blue. X-ray of skull showed multiple worm-eaten bones and radiological evidence of hydrocephalus. X-ray of the legs showed marked bowing as previously noted in the film taken during pregnancy and fracture of right femur. He made satisfactory progress and on discharge on 26.2.68 was weighing 22.0 g. At follow up there was no major difficulty although it was noted that weight gain had been much slower than the unaffected twin. At the age of 18 months he has not stood nor walked. Haemoglobin genotype = AS.

The twin brother weighed 3100 g at birth with a head circumference of 33.6 cm. Examination of all the systems was completely normal. He became moderately asthmatic in the first week of life but he required no special treatment. On discharge he weighed 4000 g. The twin died suddenly from unknown cause but suspected to be a complication of acute cell disease at a time when full investigation of the twins for zygosity was being arranged.

**Family history.** Mother = 160 cm tall. Pregnancy was normal and the reason for hospital delivery was twin pregnancy and the suspected abnormality in one of the twins. There was no physical defect detected on examination and the Haemoglobin genotype = AS.

Father is a short man (height 150.93 cm) with a relatively large head and faint bluish sclerae with brownish pigmentation. He gave no history of fractures at any time. Haemoglobin genotype is also reported as AS.

## DISCUSSION

Osteogenesis imperfecta is infrequently seen in Nigeria. In a five year period at the University College Hospital Ibadan two definite cases have been previously diagnosed. One was a four-year-old male child who presented with multiple fractures of tibia and fibula with gross deformity, dwarfing and frontal bossing. He had no bluish sclerae. The second was a one-week-old male baby who presented at

birth with limb deformities and blue sclerae. Clinical and radiological examinations established osteogenesis imperfecta. This baby died suddenly at the age of 30 days.

The cases reported here are one of two pairs of like-sexed twins but no pair of twins could be more dissimilar in appearance. However, complete haematological and other investigations to determine zygosity were not possible in these present case reports. The limited available information would suggest that the parents (mother in 1st father in 2nd) are heterozygous carriers and the condition in the babies was transmitted as a recessive characteristic.

The work of Simons (8) however showed that majority of reported cases of osteogenesis imperfecta were due to dominant autosomal genes but there are cases which do not fall into this type of inheritance. Into the latter group fall cases where the disease have missed a generation or there are no demonstrable osteogenesis imperfecta in the parents or other ribs. These latter cases can be explained on the basis of complete suppression in one generation or the mutation of the gene in the offspring.

There are few reports of osteogenesis imperfecta in twins. Zerkow *et al* (10) reported for the first time the condition in dizygotic twins both of whom were affected. The parents were free of any trait of the disease. Other reports have been in siblings. Seedorf (7) reported 180 affected members of 55 Danish families and in six of these both parents were normal and found seven cases of the congenital type. He concluded from this study that the condition was inherited as a result of a dominant mutant gene.

Heys *et al* (5) emphasised the association of osteogenesis imperfecta and odontogenesis imperfecta which existed in 18 families studied. There were only 2 cases of the congenital form in this series. Freda *et al* (3) in a review of the literature found 90 cases to which they added 16 of their own. Sarma (6) encountered one case among 543 abnormal babies. Chawla (2) reported one family in which there were

## CASE REPORT

### OSTEOGENESIS IMPERFECTA CONGENITA IN ONE OF TWINS

O SERIKI

*From the Department of Paediatrics University College Hospital  
Ibadan Nigeria*

Osteogenesis imperfecta is characterised by increased fragility of bones, spontaneous or slight traumatic fractures. Associated features are blue sclerae and otosclerosis in adult life. There are various types of the condition based on time of onset and severity.

The congenital type was first described by Vrolik in 1849 (9). This type presents a characteristic clinical picture and represents the extreme example of the disease. In this type fracture deformities may occur in utero or the bones may break during parturition. Many victims of the disease are stillborn or succumb shortly after birth.

The mode of inheritance of this condition has not been clearly defined. Goldfarb & Ford (4) reported two consecutive female siblings with congenital osteogenesis imperfecta, one of whom lived to the age of 5 months and the other beyond 5 months. Both had blue sclerae and evidence of fractures in utero with further fractures occurring after birth. Awwaad & Reda (1) described two similar cases of congenital osteogenesis imperfecta in one sibship. More recently Zeitoun *et al* (10) described the same condition in dizygotic twins. The two infants survived for 2 1/2 months and 4 months respectively and both died from bronchopneumonia.

Further examples of osteogenesis imperfecta congenita in one of two sets of twins are now

reported. This is probably the first report of this condition from West Africa.

#### *Case I*

Twin I (U.C.H. No. 196998) a female was admitted to University College Hospital (U.C.H.) at the age of 17 hours on account of her small size and limb deformities. She was born at home on a farm after an apparently normal pregnancy and labour.

Physical examination showed a small premature baby weighing 1374 g. The head was globular and showed very marked softening of the cranial bones. The anterior and posterior fontanelles were wide and slightly tense. The eyes were bulging and the sclerae blue. There were multiple fracture deformities of the four limbs giving the infant a characteristic appearance. There was no other abnormality in all the systems.

Radiographs showed radiological features of osteogenesis imperfecta with fractures of all limbs and ribs.

Laboratory findings were: Serum calcium 8.8 mg per 100 ml, inorganic phosphate 5.9 mg per 100 ml, alkaline phosphatase 19 k.A. units, Serum urea 24 mg per 100 ml and normal serum electrolytes.

Twin II weighed 1322 g on admission. Clinical and radiological examinations were completely normal. She thrived well and on discharge thirty days after had gained 651 g.

*Family history* The mother was a young lady with the only abnormal finding of bluish sclerae. The father was not available for examination but detailed enquiries from the wife did not suggest abnormality in the father or other members of the family. A 3 year old sibling of the case was healthy and normal.

*Management* The baby was nursed in an incubator and fed by a naso-gastric tube. She had a course of prophylactic penicillin and anti-tetanus serum. Progress was satisfactory until twenty-one days after admission when she developed a cold but examination

of the chest showed no abnormality. A nasal swab was taken and she was started on a course of tetracycline. Two days after the onset of the upper respiratory infection she suddenly collapsed and died. Necropsy confirmed the diagnosis of osteogenesis imperfecta and death was considered to be due to the presence of a large subdural haematoma.

#### Case 2

**P. O. male** one of twins was born in UCH on 31.12.67 at 36 weeks to a young primigravida. He is the second twin and weighed 1800 g at birth. The skull showed asymmetry with multiple defects and large anterior fontanelle. The lower legs were short and bowed and there was evidence of fractures of both tibiae and fibulae. The sclerae were blue. X ray of skull showed multiple wormian bones and radiological evidence of hydrocephalus. X ray of the legs showed marked bowing as previously noted in the film taken during pregnancy and fracture of right femur. He made satisfactory progress and on discharge on 26.2.68 was weighing 2270 g. At follow up there was no major difficulty although it was noted that weight gain had been much slower than the unaffected twin. At the age of 18 months he has not stood nor walked. Haemoglobin genotype is AS.

The twin brother weighed 3100 g at birth with a head circumference of 35.6 cm. Examination of all the systems was completely normal. He became moderately sclerotic in the first week of life but he required no special treatment. On discharge he weighed 4000 g. This twin died suddenly from unknown cause but suspected to be a complication of sickle cell disease at a time when full investigation of the twins for zygosity was being accepted.

**Family history.** Mother is 160 cm tall. Pregnancy was normal and the reason for hospital delivery was twin pregnancy and the suspected abnormality in one of the twins. There was no physical defect detected on examination and the Haemoglobin genotype is AS.

Father is a short man (height 150.93 cm) with a relatively large head and front bluish sclerae with brownish pigmentation. He gave no history of fractures at any time. Haemoglobin genotype is also reported as AS.

#### DISCUSSION

Osteogenesis imperfecta is infrequently seen in Nigeria. In a five year period at the University College Hospital Ibadan two definite cases have been previously diagnosed. One was a four year-old male child who presented with multiple fractures of tibia and fibula with gross deformity, dwarfing and frontal bossing. He had no bluish sclerae. The second was a one week-old male baby who presented at

birth with limb deformities and blue sclera. Clinical and radiological examinations established osteogenesis imperfecta. This baby died suddenly at the age of 30 days.

The cases reported here are one of two pairs of like sexed twins but no pair of twins could be more dissimilar in appearance. However complete haematological and other investigations to determine zygosity were not possible in these present case reports. The limited available information would suggest that the parents (mother in 1st, father in 2nd) are heterozygous carriers and the condition in the babies was transmitted as a recessive characteristic.

The work of Smårs (8) however showed that majority of reported cases of osteogenesis imperfecta were due to dominant autosomal gene but there are cases which do not fall into this type of inheritance. Into the latter group fall cases where the disease have missed a generation or there are no demonstrable osteogenesis imperfecta in the parents or other sibs. These latter cases can be explained on the basis of complete suppression in one generation or the mutation of the gene in the offspring.

There are few reports of osteogenesis imperfecta in twins. Zetoun *et al* (10) reported for the first time the condition in dizygotic twins both of whom were affected. The parents were free of any trait of the disease. Other reports have been in siblings. Seedorff (7) reported 180 affected members of 55 Danish families and in six of these both parents were normal and found seven cases of the congenital type. He concluded from this study that the condition was inherited as a result of a dominant mutant gene.

Heys *et al* (5) emphasized the association of osteogenesis imperfecta and odontogenesis imperfecta which existed in 18 families studied. There were only 2 cases of the congenital form in this series. Freda *et al* (3) in a review of the literature found 90 cases to which they added 16 of their own. Sarma (6) encountered one case among 543 abnormal babies. Chawla (2) reported one family in which there were



## CASE REPORT

### OSTEOGENESIS IMPERFECTA CONGENITA IN ONE OF TWINS

O SERIJI

*From the Department of Paediatrics University College Hospital  
Ibadan Nigeria*

Osteogenesis imperfecta is characterised by increased fragility of bones, spontaneous or slight traumatic fractures. Associated features are blue sclerae and otosclerosis in adult life. There are various types of the condition based on time of onset and severity.

The congenital type was first described by Vrolik in 1849 (9). This type presents a characteristic clinical picture and represents the extreme example of the disease. In this type fracture deformities may occur in utero or the bones may break during parturition. Many victims of the disease are stillborn or succumb shortly after birth.

The mode of inheritance of this condition has not been clearly defined. Goldfarb & Ford (4) reported two consecutive female siblings with congenital osteogenesis imperfecta, one of whom lived to the age of 5 months and the other beyond 5 months. Both had blue sclerae and evidence of fractures in utero with further fractures occurring after birth. Awwaad & Reda (1) described two similar cases of congenital osteogenesis imperfecta in one sibship. More recently Zentoun *et al* (10) described the same condition in dizygotic twins. The two infants survived for 2 1/2 months and 4 months respectively and both died from bronchopneumonia.

Further examples of osteogenesis imperfecta congenita in one of two sets of twins are now

reported. This is probably the first report of this condition from West Africa.

#### Case 1

Twin I (U.C.H. No 196998) a female was admitted to University College Hospital (U.C.H.) at the age of 17 hours on account of her small size and limb deformities. She was born at home on a farm after an apparently normal pregnancy and labour.

Physical examination showed a small premature baby weighing 1374 g. The head was globular and showed very marked softening of the cranial bones. The anterior and posterior fontanelles were wide and slightly tense. The eyes were bulging and the sclerae blue. There were multiple fracture deformities of the four limbs giving the infant a characteristic appearance. There was no other abnormality in all the systems.

Radiographs showed radiological features of osteogenesis imperfecta with fractures of all limbs and ribs.

Laboratory findings were: Serum calcium 8.8 mg per 100 ml, inorganic phosphate 5.9 mg per 100 ml, alkaline phosphatase 19 K.A. units, Serum urea 24 mg per 100 ml and normal serum electrolytes.

Twin II weighed 1322 g on admission. Clinical and radiological examinations were completely normal. She thrived well and on discharge thirty days after had gained 633 g.

**Family history.** The mother was a young lady with the only abnormal finding of bluish sclerae. The father was not available for examination but detailed enquiries from the wife did not suggest abnormality in the father or other members of the family. A 3 year old sibling of the case was healthy and normal.

**Management.** The baby was nursed in an incubator and fed by a naso-gastric tube. She had a course of prophylactic penicillin and anti-tetanus serum. Progress was satisfactory until twenty one days after admission when she developed a cold but examination

of the chest showed no abnormality. A nasal swab was taken and she was started on a course of tetracycline. Two days after the onset of the upper respiratory infection she suddenly collapsed and died. Necropsy confirmed the diagnosis of osteogenesis imperfecta and death was considered to be due to the presence of a large subdural haematoma.

#### Case 2

B O male one of twins was born in UCH on 31.12.67 at 34 weeks to a young primigravida. He is the second twin and weighed 1800 g at birth. The skull showed asymmetry with multiple defects and large anterior fontanelle. The lower legs were short and bowed and there was evidence of fractures of both tibiae and fibulae. The sclerae were blue. X ray of skull showed multiple wormian bones and radiological evidence of hydrocephalus. X ray of the legs showed marked bowing as previously noted in the film taken during pregnancy and fracture of right femur. He made satisfactory progress and on discharge on 25.2.68 was weighing 2270 g. At follow up there was no major difficulty although it was noted that weight gain had been much slower than the unaffected twin. At the age of 18 months he has not stood nor walked. Haemoglobin genotype is AS.

The twin brother weighed 3100 g at birth with a head circumference of 35.6 cm. Examination of all the systems was completely normal. He became moderately sclerotic in the first week of life but he required no special treatment. On discharge he weighed 4000 g. The twin died suddenly from unknown cause but suspected to be a complication of meckel cell disease at time when full investigation of the twins for zygosity was being arranged.

**Family history.** Mother is 160 cm tall. Pregnancy was normal and the reason for hospital delivery was twin pregnancy and the suspected abnormality in one of the twins. There was no physical defect detected on examination and the Haemoglobin genotype is AS.

Father is a short man (height 130.95 cm) with a relatively large head and faint bluish sclerae with brownish pigmentation. He gave no history of fractures at any time. Haemoglobin genotype is also reported as AS.

#### DISCUSSION

Osteogenesis imperfecta is infrequently seen in Nigeria. In a five year period at the University College Hospital Ibadan two definite cases have been previously diagnosed. One was a four year-old male child who presented with multiple fractures of tibia and fibula with gross deformity, dwarfing and frontal bossing. He had no bluish sclerae. The second was a one week-old male baby who presented at

birth with limb deformities and blue sclera. Clinical and radiological examinations established osteogenesis imperfecta. This baby died suddenly at the age of 30 days.

The cases reported here are one of two pairs of like sexed twins but no pair of twins could be more dissimilar in appearance. However complete haematological and other investigations to determine zygosity were not possible in these present case reports. The limited available information would suggest that the parents (mother in 1st (father in 2nd) are heterozygous carriers and the condition in the babies was transmitted as a recessive characteristic.

The work of Sanluis (8) however showed that majority of reported cases of osteogenesis imperfecta were due to dominant autosomal genes but there are cases which do not fall into this type of inheritance. Into the latter group fall cases where the disease have missed a generation or there are no demonstrable osteogenesis imperfecta in the parents or other sibs. These latter cases can be explained on the basis of complete suppression in one generation or the mutation of the gene in the offspring.

There are few reports of osteogenesis imperfecta in twins. Zetoun *et al* (10) reported for the first time the condition in dizygotic twins both of whom were affected. The parents were free of any trait of the disease. Other reports have been in siblings. Seedorff (7) reported 180 affected members of 55 Danish families and in six of these both parents were normal and found seven cases of the congenital type. He concluded from this study that the condition was inherited as a result of a dominant mutant gene.

Heys *et al* (5) emphasized the association of osteogenesis imperfecta and odontogenesis imperfecta which existed in 18 families studied. There were only 2 cases of the congenital form in this series. Freda *et al* (3) in a review of the literature found 90 cases to which they added 16 of their own. Sarma (6) encountered one case among 543 abnormal babies. Chawla (2) reported one family in which there were

four affected siblings—3 males and 1 female. Remaining 3 siblings as well as the parents were unaffected. These reports indicate that there is a genetic factor in the aetiology of this condition and the majority of cases are inherited as autosomal dominant although other mode of inheritance could not be ruled out.

### SUMMARY AND CONCLUSION

Cases of osteogenesis imperfecta congenita in one of twins are reported. This condition is uncommon in single births and more so among twin deliveries. It is invariably fatal and this will partially explain its rarity as many are still born or die shortly after. It is also possible that the incidence of the disease will vary from one geographical area to another depending on the frequency of the particular gene in the population. A more detailed family studies and investigation of new cases in twins may give more information on the question of inheritance in the African population.

### REFERENCES

- 1 Awward S & Reda M. Osteogenesis imperfecta. *Arch Pediat* 77: 280 1960.
- 2 Chawla S. Intrauterine osteogenesis imperfecta in four siblings. *Brit Med J* 1: 99 1964.
- 3 Freda V J, Vosburgh G J & Diliberti C.

- Osteogenesis imperfecta congenita: a presentation of 16 cases and review of the literature. *Obstet Gynaec* 18: 535 1961.
- 4 Goldfarb A A & Ford D. Osteogenesis imperfecta congenita in consecutive siblings. *J Pediatr* 44: 264 1954.
- 5 Heya F M, Blattner H J & Robinson H I. G. Osteogenesis imperfecta and odonto-genesis imperfecta: clinical and genetic aspects in eighteen families. *J Pediatr* 56: 234 1960.
- 6 Sarma V. A case of intrauterine osteogenesis imperfecta. *Br Med J* 11: 1856 1960.
- 7 Seedorff K. E. *Osteogenesis imperfecta. A study of clinical features and heredity. Based on 5 Danish families comprising 180 affected persons.* Universitetsforlaget Aarhus 1949 vol 20.
- 8 Småns G. *Osteogenesis imperfecta in Sweden. Clinical genetic epidemiological and socio-medical aspects.* Scandinavian Univ Books Stockholm 1961.
- 9 Vrolik W cited by J Bell. *Blue sclerotics and fragility of bone in Treasury of Human Inheritance* 2: III Francis Galton Laboratory for Natural Eugenics University of London 1928.
- 10 Zentoun M M, Ibrahim A H & Kassem A S. Osteogenesis imperfecta congenita in dizygotic twins. *Arch Dis Child* 38: 289 1963.

Submitted Sept. 22 1969

Accepted Oct. 9 1969

Department of Paediatrics  
University College Hospital  
Ibadan  
Nigeria

**Key words:** Osteogenesis imperfecta congenita; twins; inheritance.

## CASE REPORT

### GROWTH HORMONE RESPONSE TO INSULIN INDUCED HYPOGLYCEMIA IN A BOY WITH DIABETES INSIPIDUS AND SHORT STATURE BEFORE AND AFTER TREATMENT WITH VASOPRESSIN

P OLIN

*From the Department of Paediatrics, Krasimirovskan Läkarska Barneakademik  
Karolinska Institute, Stockholm, Sweden*

The problems of evaluating the plasma growth hormone (GH) response to insulin induced by hypoglycemia in children of short stature are now well recognized (7). The patient with short stature and diabetes insipidus that is presented here adds some pertinent data in this context.

## CASE REPORT

A 15.58 year old boy was referred from the school physician because of stunted growth since the age of 12 years. His father and elder brother 22 years old were 172 cm tall and his mother was 166 cm. No endocrine disorders were known in the non-consanguineous family. During the last four years preceding the admittance to the hospital the patient had been easily tired but managed very well at school. His skin became dry. He did not sweat. He was more sensitive to cold temperatures. He had been drinking excessively and voided several times every night. The analysis of the growth data obtained from the school records and at the first visit revealed a marked retardation in development.

As shown in Fig. 1 his height was normal around -1 s.d. to the age of 11.6 years. The height increment was 4.5 to 6 cm/year. During the next 3.4 years the increment was only 1.6 to 1 cm/year. A slightly increased growth rate of 3.1 cm/year was noted during half a year preceding the admittance.

#### Physical examination

At the chronological age of 15.58 his height was 146 cm (3 s.d.) span 146 cm, lower segment 74 cm. Weight 35.5 kg (average for height). There was a slight development of the secondary sex characters.

Testes Penn and scrotum was not stimulated. Pubic hair was sparse grade II according to Greulich (1). The testes were descended and each were 25 × 15 mm. The skin was dry and mottled and the subcutaneous tissue was increased. Eye movements and vision were normal. The ankle jerk relaxation time was not prolonged. Deep tendon reflexes were present.

#### Laboratory data

Routine hematology was normal. Some data regarding the pituitary function are given in Table 1. The results were consistent with an antidiuretic hormone (ADH) deficiency. The thyroid function tests were essentially normal although a mild deficiency of thyrotrophic hormone (TSH) could not be excluded. The normal response of urinary 17-OH-corticosteroids to metyrapone indicated a normal adrenocorticotrophic (ACTH) adrenal function. The urinary gonadotrophins were low but the boy was immaturely judged by clinical criteria such as the size of the testes, the presence of pubic hair and the slight increase in growth rate. Radiological examinations of the skull and sella were normal. An EEG and a pneumoencephalogram including repeated tracings of the visual field were normal. The skeletal age was 12 years according to the atlas of Greulich & Pyle (3).

#### Clinical course

A diagnosis of combined ADH, GH and possibly TSH deficiency was suspected (Data on plasma GH is presented under Special studies). Treatment with thyroxine and a nasal preparation of vasopressin was given for six months. As shown in Fig. 1 the growth rate increased. When human growth hormone (gift from Dr Roos, Uppsala, Sweden) was added to the

four affected siblings—3 males and 1 female. Remaining 3 siblings as well as the parents were unaffected. These reports indicate that there is a genetic factor in the aetiology of this condition and the majority of cases are inherited as autosomal dominant although other mode of inheritance could not be ruled out.

## SUMMARY AND CONCLUSION

Cases of osteogenesis imperfecta congenita in one of twins are reported. This condition is uncommon in single births and more so among twin deliveries. It is invariably fatal and this will partially explain its rarity as many are still born or die shortly after. It is also possible that the incidence of the disease will vary from one geographical area to another depending on the frequency of the particular gene in the population. A more detailed family studies and investigation of new cases in twins may give more information on the question of inheritance in the African population.

## REFERENCES

- 1 Awwaad S & Reda M. Osteogenesis imperfecta. *Arch Pediat* 77: 280 1960.
- 2 Chawla S. Intrauterine osteogenesis imperfecta in four siblings. *Brit Med J* 1: 99 1964.
- 3 Freda V I, Vosburgh G I & Diliberti C. Osteogenesis imperfecta congenita: a presentation of 16 cases and review of the literature. *Obstet Gynaec* 18: 535 1961.
- 4 Goldfarb A A & Ford D. Osteogenesis imperfecta congenita in consecutive siblings. *J Pediatr* 44: 264 1954.
- 5 Heys F M, Blattner R J & Robinson H L G. Osteogenesis imperfecta and odontogenesis imperfecta: clinical and genetic aspects in thirteen families. *J Pediatr* 56: 234 1960.
- 6 Sarma V. A case of intrauterine osteogenesis imperfecta. *Brit Med J* 11: 1856 1960.
- 7 Sedorff J. E. *Osteogenesis imperfecta. A study of clinical features and heredity. Based on 55 Danish families comprising 180 affected persons.* Universitetsforlaget, Århus 1949, vol 20.
- 8 Smårs G. Osteogenesis imperfecta in Sweden. Clinical, genetic, epidemiological and sociomedical aspects. Scandinavian Univ Books, Stockholm 1961.
- 9 Vrolik W. cited by J Bell. *Blue sclerotics and fragility of bone* in Treasury of Human Inheritance 2. III Francis Galton Laboratory for National Eugenics, University of London 1928.
- 10 Zeitoun M M, Ibrahim A H & Hassan A S. Osteogenesis imperfecta congenita in dizygotic twins. *Arch Dis Child* 38: 289 1963.

Submitted Sept 22 1969

Accepted Oct 9 1969

Department of Paediatrics  
University College Hospital  
Ibadan  
Nigeria

**Key words:** Osteogenesis imperfecta congenita, twins, inheritance.

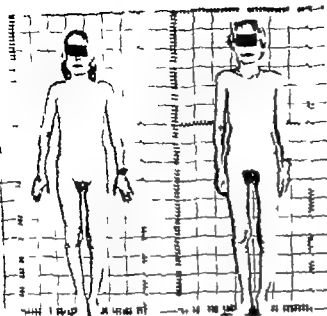


Fig 2 The patient at the first admission when he was 15.85 years old (left). The skin was pale and dry. Significant pubertal development is evident. The appearance of the patient one year later at the age of 16.85 years (right) when he had been on treatment with thyroxine and growth hormone for 4 months. The subcutaneous tissue is markedly reduced. The growth increment 5 cm/year. The pubertal development was more advanced.

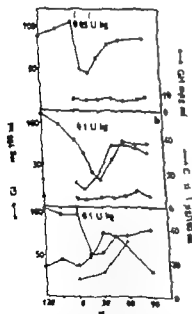


Fig 3 Insulin tolerance tests. (a) At the age of 15.83 no therapy was given. The plasma GH response to hypoglycemia is blunted. (b) After treatment with thyroxine 0.1 mg/day for three months the plasma GH response is inadequate. (c) At the age of 16.83 when thyroxine (0.1 mg) and GH (5 U) had been given 5 U daily for ten days. The plasma growth hormone response is normalized.

growth hormone was discontinued 20 days before this study. The boy was on a nitrogen constant diet for 5 days before the test. As shown in the figure the basal plasma growth hormone levels were higher than in the earlier tests and the response to hypoglycemia was marked. Plasma cortisol also increased adequately.

### COMMENTS

The striking feature in this patient is that the treatment with vasopressin normalized a previously abnormal growth hormone response to insulin induced hypoglycemia. Untreated or poorly controlled diabetes insipidus due to vasopressin insufficiency in childhood is suggested to cause an inadequate caloric intake followed by hypocaloric dwarfism (6). It is not clear whether the so called hypocaloric dwarfism is caused by a general depletion of nutrients such as protein to the growing tissues or if there is insufficient synthesis or release of growth hormone. The data from this patient favours that vasopressin is necessary to allow an adequate release of growth hormone. The absence of other signs of malnutrition such as anaemia, low serum protein and depletion of body fat makes an inhibition of growth hor-

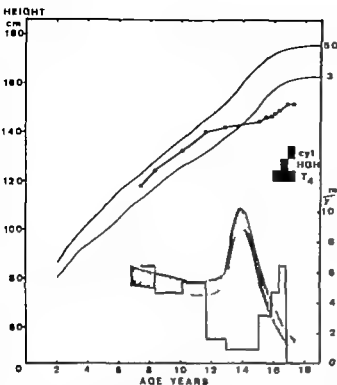


Fig 1 Growth chart. The height in centimeter is plotted against chronological age in the upper part of the figure. In the lower part the height velocity is given in cm/year. The growth data were obtained from the school record up to the age of 15.8. Thereafter the height was measured in the morning on visits to the hospital. Each of these measurements represent the mean of two readings. The data are plotted on the growth chart of Tanner *et al* (5). The 50th and 3rd percentile for height is given. The shaded area represents the growth velocity curve for longitudinally followed boys of average height according to Tanner *et al* (5). The black bar indicates periods of therapy: CYT—Cytostatic treatment with vincoblastine i.v. 0.15 mg/kg per week and prednisolone 5 mg  $\times$  4; GHG—Human growth hormone 2 mg twice weekly; T—thyroxine 0.2 mg daily.

regime for another period of 5 months the growth increment was only slightly more augmented.

The patient was readmitted at the age of 16.85 years to evaluate the preliminary diagnosis. His height was 151.5 cm, weight 34.6 kg. The subcutaneous fat was markedly reduced. The skin was normal. The testes were 35  $\times$  20 mm resp. 30  $\times$  20 mm. Pubic hair grade III and axillary hair grade II. It was realized that the boy had only sporadically utilized the nasal preparation of vasopressin. Adequate substitution therapy with pitressin tannate in oil was given and plasma growth hormone was again studied as described below. Before any further change in the therapy was undertaken the boy developed symptoms of a cervical neuropathy. A radiological examination showed multiple destructions of the fifth cervical vertebra and multiple infiltrates in both lungs. No other skeletal defect was observed. The diagnosis of Hand-

Schüller-Christian's disease was established by cytological examination of an aspiration biopsy from the destroyed vertebra (performed by Dr S. Franzen, Stockholm). The patient did not grow at all during the combined treatment with vincoblastine and corticosteroids that followed (Fig. 1).

## SPECIAL STUDIES

Intravenous insulin tolerance tests were performed after fasting over night when the patient was still in bed. A catheter was inserted into a cubital vein two hours before the insulin was injected. The catheter was kept open throughout the test by a slow infusion of normal saline. All blood samples were drawn from the catheter. Plasma growth hormone was determined by a double antibody radioimmunoassay (2). Plasma cortisol was determined fluorometrically and blood glucose by a glucose oxidase method. The insulin dose was 0.05 U/kg body weight in the first test and in the following tests 0.1 U/kg. In all tests the minimum blood sugar level was less than 50% of the fasting level. The first test (Fig. 3a) was performed at the age of 15.85 years before any treatment was given. The plasma growth hormone level did not change during the test. The second test (Fig. 3b) was performed when the patient was on thyroxine 0.2 mg daily since three months. Plasma growth hormone did not change but for one value of 9.8 mUg/ml at 75 min after the insulin injection. Plasma cortisol increased markedly. The third test (Fig. 3c) was performed at the age of 16.85 when the patient had received pitressin tannate in oil 5 U i.m. daily for ten days and thyroxine 0.2 mg daily for one year. Treatment with human

Table 1 Laboratory data regarding the function of the pituitary gland

Urinary 17 hydroxycorticosteroids	18
mg/24 hours	51
After metyrapone 500 mg 4	5.5-6.0
PBI $\mu$ g/100 ml	83
$T_4$ resin uptake (normal range 85-125)	20
$^{125}I$ uptake after 24 hours	51
After thyrotropin 3 U s.c. i.m.	130-310
Urinary osmolality mOsmol/kg	5-7
Urinary volume liters/24 h	<6.5
Urinary gonadotrophins mouse units	

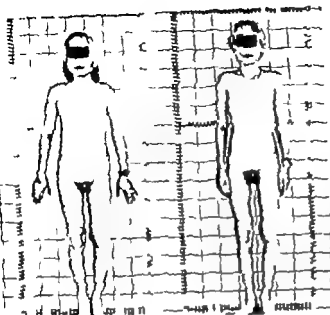


Fig 2 The patient at the first adult intake when he was 15.85 years old (left). The skin was pale and dry. Stage 1 pubertal development is evident. The appearance of the patient one year later at the age of 16.85 years (right) when he had been on treatment with thyroxine and growth hormone for 5 months. The subcutaneous tissue is markedly reduced. The growth increment 5 cm/year. The pubertal development was more advanced.

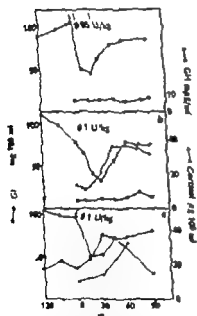


Fig 3 IV insulin tolerance tests. (a) At the age of 15.85 no therapy was given. The plasma GH response to hypoglycaemia is blunted. (b) After treatment with thyroxine 0.2 mg/day for three months the plasma GH response is inadequate. (c) At the age of 16.85 when prednisolone tablets in oil had been given 5 U on study for ten days. The plasma growth hormone response is normalized.

growth hormone was discontinued 20 days before this study. The boy was on a nitrogen constant diet for 5 days before the test. As shown in the figure the basal plasma growth hormone levels were higher than in the earlier tests and the response to hypoglycaemia was marked. Plasma cortisol also increased adequately.

### COMMENTS

The striking feature in this patient is that the treatment with vasopressin normalized a previously abnormal growth hormone response to insulin induced hypoglycaemia. Untreated or poorly controlled diabetes insipidus due to vasopressin insufficiency in childhood is suggested to cause an inadequate caloric intake followed by hypocaloric dwarfism (6). It is not clear whether the so called hypocaloric dwarfism is caused by a general depletion of nutrients such as protein to the growing tissues or if there is insufficient synthesis or release of growth hormone. The data from this patient favours that vasopressin is necessary to allow an adequate release of growth hormone. The absence of other signs of malnutrition such as anaemia, low serum protein and depletion of body fat makes an inhibition of growth hor-



hormone synthesis improbable although this mechanism cannot be excluded. The risks in using a nasal preparation of vasopressin to control diabetes insipidus in childhood (6) was unfortunately confirmed. Blunted plasma growth hormone responses to insulin induced hypoglycemia or arginine infusion in patients with diabetes insipidus have been reported by Landon *et al* 1966 (4) and Yuolton *et al* 1969 (7). The authors do not state if adequate substitution therapy with ADH was given. The data from this patient again demonstrated the fallacy of establishing a diagnosis of growth hormone deficiency on the basis of an absent growth hormone response to insulin induced hypoglycemia before other interfering factors are excluded. Hypothyroidism, cortisol treatment and malnutrition among other circumstances may blunt the growth hormone response. To this list uncompensated diabetes insipidus should be added.

### SUMMARY

A 16-year old boy with Hand-Schüller-Christian's disease with diabetes insipidus and short stature is presented. The plasma growth hormone response to insulin induced hypoglycemia was reduced at two occasions but was normalized after adequate substitution with vasopressin. The finding is discussed in connection to the pathogenesis of growth retardation in patients with diabetes insipidus.

### ACKNOWLEDGEMENT

The radioimmunoassay of growth hormone was kindly performed by Dr E. Cerasi, Stockholm.

### REFERENCES

- Andersson E. Skeletal maturation of Danish school children in relation to height, sexual development and social conditions. *Acta Paediatr Scand Suppl* 185: 32, 1968.
- Cerasi E, Della Casa L., Luft R. & Roove A. Determination of human growth hormone (HGH) in plasma by a double antibody radioimmunoassay. *Acta Endocr* 53: 101, 1966.
- Greulich W. W. & Pyle I. S. *Radiographic atlas of skeletal development of the hand and wrist*. Stanford, California: Stanford University Press, 1959.
- Landon J., Greenwood F. C., Stampard T. C. G. & Wynn W. The plasma sugar free fatty acids, cortisol and growth hormone response to insulin and the comparison of this procedure with other tests of pituitary and adrenal function. II. In patients with hypothalamic or pituitary dysfunction or anorexia nervosa. *J Clin Invest* 45: 437, 1966.
- Tanner J. M., Whitehouse R. M. & Takashi M. Standards from birth to maturity for height, weight, height velocity and weight velocity. *Arch Dis Child* 41: 454, 1966.
- Vest, M., Talbot N. B. & Crawford J. D. Hypoparathyroid dwarfism and hydrocephalus in diabetes insipidus. *Am J Dis Child* 105: 175, 1963.
- Yuolton R., Kaplan S. L. & Grumbach M. M. Growth and growth hormone. IV. Limitations of the growth hormone response to insulin and arginine and of the immunoreactive insulin response to arginine in the assessment of growth hormone deficiency in children. *Pediatrics* 43: 989, 1969.

Submitted Nov. 11, 1969

Accepted Nov. 25, 1969

Dept. of Paediatrics  
St. Gorans Sjukhus  
St. Goransgatan 143  
S-112 51 Stockholm  
Sweden

**Key words:** Diabetes insipidus, Hand-Schüller-Christian's disease, growth hormone response, vasopressin treatment, short stature.

PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE DANISH PAEDIATRIC SOCIETY

Meeting April 9 1969

*The future of paediatrics in Denmark* Discus-  
sion

Meeting May 28 1969

H Krentzfeldt *Information concerning family  
advisory activities of the services for children  
and young people*

A Wilken Jensen *Allergic infantile alimentary  
purpura*

K Wilken Jensen & J C Melchior *Allergy to  
foodstuffs and neurological symptoms*

J Møller *Haemolytic uraemic syndrome*

A report is given of a syndrome occurring with increasing frequency. Briefly the syndrome consists of an acute condition with development of haemolytic anaemia and uraemia accompanied by thrombocytopenia and a great variety of functional disturbances in other organs including the brain. The syndrome has been described exclusively in children but there appears to be a certain justification for regarding it as a variant of Moschowitz syndrome or thrombotic thrombocytopenic purpura which has been described predominantly in adults.

A fatal case in a boy aged eight years is described. Treatment with dialysis, blood transfusions, glucocorticoids and late after the commencement of the illness with heparin was without effect.

Very little is known of the cause of the condition. It is occasionally observed in siblings although this is rare and geographic accumulation is recognized. Some authors have ob-

served the occurrence of an ultrafiltrable agent and an increase of antibodies against this substance.

All of the symptoms appear to be explained by acute intra vascular coagulation which may be demonstrated *inter alia* by renal biopsy in the acute phase of the disease. The process takes place mainly in the capillaries and the symptoms can be explained by the formation of microinfarcts. The thrombocytopenia is explained by absorption of the thrombocytes in the microthrombi. Precipitated fibrin and possibly damaged vascular endothelium are considered to provoke the accompanying haemolysis.

Treatment is symptomatic and consists of dialysis, transfusions and if necessary anti-epileptic and blood pressure lowering therapy. Most important is the treatment with heparin which has frequently resulted in dramatic improvement if undertaken at an early phase of the disease. Glucocorticoids should probably be employed with care as these substances are known to facilitate provocation of the Schwartzmann phenomenon. There is a surprising similarity between haemolytic uraemic disease and this purely experimental phenomenon. The syndrome should probably be regarded as a localized Schwartzmann phenomenon.

more synthesis improbable although this mechanism cannot be excluded. The risks in using a nasal preparation of vasopressin to control diabetes insipidus in childhood (6) was unfortunately confirmed. Blunted plasma growth hormone responses to insulin induced hypoglycemia or arginine infusion in patients with diabetes insipidus have been reported by Landon *et al* 1966 (4) and Yuolton *et al* 1969 (7). The authors do not state if adequate substitution therapy with ADH was given. The data from this patient again demonstrated the fallacy of establishing a diagnosis of growth hormone deficiency on the basis of an absent growth hormone response to insulin induced hypoglycemia before other interfering factors are excluded. Hypothyroidism, cortisol treatment and malnutrition among other circumstances may blunt the growth hormone response. To this list uncompensated diabetes insipidus should be added.

### SUMMARY

A 16-year-old boy with Hand-Schüller-Christian's disease with diabetes insipidus and short stature is presented. The plasma growth hormone response to insulin induced hypoglycemia was reduced at two occasions but was normalized after adequate substitution with vasopressin. The finding is discussed in connection to the pathogenesis of growth retardation in patients with diabetes insipidus.

### ACKNOWLEDGEMENT

The radioimmunoassay of growth hormone was kindly performed by Dr E. Cerasi, Stockholm.

### REFERENCES

- 1 Andersen E: Skeletal maturation of Danish school children in relation to height, sexual development and social conditions. *Acta Paediatr Scand Suppl* 183: 32, 1968.
- 2 Cerasi E, Della Casa L, Luft H & Rooven A: Determination of human growth hormone (HGH) in plasma by a double antibody radioimmunoassay. *Acta Endocr* 53: 101, 1966.
- 3 Greulich W W & Pyle I S: *Radiographic atlas of skeletal development of the hand and wrist*. Stanford, California: Stanford University Press, 1959.
- 4 Landon J, Greenwood F C, Stampard T C, G & Wynn W: The plasma sugar free fatty acids, cortisol and growth hormone response to insulin and the comparison of this procedure with other tests of pituitary and adrenal function. In patients with hypothalamic or pituitary dysfunction or anorexia nervosa. *J Clin Invest* 45: 437, 1966.
- 5 Tanner J M, Whitehouse R M & Takaishi M: Standards from birth to maturity for height, weight, height velocity and weight velocity. *Arch Dis Child* 41: 454, 1966.
- 6 Vest M, Talbot N B & Crawford J D: Hypocaloric dwarfism and hydrocephrosis in diabetes insipidus. *Am J Dis Child* 105: 175, 1963.
- 7 Yuolton R, Kaplan S L & Grumbach M M: Growth and growth hormone. IV. Limitations of the growth hormone response to insulin and arginine and of the immunoreactive insulin response to arginine in the assessment of growth hormone deficiency in children. *Pediatrics* 43: 989, 1969.

Submitted Nov. 11, 1969

Accepted Nov. 25, 1969

Dept. of Paediatrics  
St. Görans Sjukhus  
S-141 86 Stockholm  
Sweden

**Key words:** Diabetes insipidus, Hand-Schüller-Christian's disease, growth hormone response, vasopressin treatment, short stature.

## Discussion

*J Vesterdal* Acidosis has been described in premature infants fed on acidified milk preparations. Feeding with cows milk has been employed since Arld's time without deficiency symptoms being described.

*H J Brandt* What is the vitamin E content?

*J Clausen* The vitamin E content is 20% lower in skim milk preparations than in the other preparations.

*E Aakhus* Are these quantities in weight per cent?

*J Clausen* The determinations are carried out by gas chromatography and thus express the content by weight.

*J Clausen & B Friis Hansen* Lipoproteins in newly born infants

Investigations of lipid metabolism in adults in recent years has revealed that the lipid absorbed from the food after transport to the liver or directly at the intestinal walls is taken up or combined partly in the chylomicron fraction and partly in the lipoprotein fractions which migrate electrophoretically in the  $\alpha$  and  $\beta$  zones (cf review by Barnstein, 1961). Further  $\alpha$ -lipoprotein occurs in serum and does not possess the above-mentioned transport function.  $\beta$ -lipoprotein consists immunologically of at least two fractions with partial immunological identity (viz  $\alpha$  2 and  $\beta$  lipoproteins) (3). By means of electrophoretic separation in albumin-containing serum the  $\beta$  lipoprotein can be separated from the  $\alpha$  2 lipoprotein which is also termed pre  $\beta$  lipoprotein which is of diagnostic significance for subdivision of anomalies of lipoproteins (7).

At birth, the newly born organism undergoes transition from parenteral to oral feeding. In agreement with the parenteral feeding of the foetus the authors found on investigation of cord blood that newly born infants lack or have a very low content of  $\beta$  and  $\alpha$  2 lipoproteins, the dominant lipid fraction in cord

blood being a fraction with an electrophoretic migration resembling  $\alpha$  1 lipoprotein.

By means of electrophoresis in albumin-containing agarose it was found, as a function of the time which had elapsed since birth, that the quantity of  $\beta$  lipoprotein rose in the course of the first two weeks. By means of quantitative radial immunodiffusion, it was demonstrated that the adult  $\beta$  lipoprotein level is not attained until the second or third week of life at the earliest and that this is influenced by individual differences.

Continued investigations will reveal whether retarded development of  $\alpha$  and  $\beta$  lipoproteins are connected with the well known of infants

## REFERENCES

- 1 Barnstein, M. *Bull Acad Suisse Sci Med* 179, 1961
- 2 Fredrickson D S & Lees, R S. *Circul* 31 321 1965
- 3 Lawrence S H & Shear F C. *Science* 15 277 1967

## Discussion

*H J Brandt* Are chylomicrons seen with this technique?

*J Clausen* No.

*J Vesterdal* Is it not possible that transport lipoproteins are present in newly born infants but do not become manifest until lipids are present?

*J Clausen* In hyperlipidemia the protein fraction varies but not the quantity of lipids which appears to contradict the above presumption.

*H Hobolth* How were these infants fed?

*J Clausen* As a rule with breast milk.

*P Krasilnikoff* Are  $\beta$  and  $\alpha$  2 lipoproteins equally suitable for lipid transport?

*J Clausen* The  $\beta$  lipoproteins are the most important and  $\alpha$  2 lipoproteins only constitute 10 per cent of the total fraction in normal adults.

P Paerregaard

with a high skin temperature after admission catheterized catheter 90- were determined g 10% glu To prevent (50 mg per per kg per two divided bicarbonate base status possible during

as by per tracheal in Acromioclavicular and falling also indicate severe respiratory (BEN) actual vent concentration Po between

S is were due the severity first 12 to full physical relaxation of ) expiratory retractions neck attacks one 0-3) as ad to respiratory insufficiency above The occur

Meeting September 25 1969

Gerald Gaull *Paediatric research and mental retardation*

Meeting October 8 1969

N J Brandt & J Philip *Hereditary Cri du Chat Syndrome*

A woman aged 33 years gave birth to an infant with typical Cri du Chat Syndrome. As she had previously had a spontaneous abortion, a family investigation was undertaken. The only abnormal finding in the phenotypically normal mother was a mosaic of normal cells and cells with deletion of the short arm of chromosome no 5. The mother became pregnant again and therapeutic abortion was undertaken. The foetus had the same chromosome abnormality as is characteristic for the Cri du Chat Syndrome. This is the first description of a mother with mosaicism for deletion of the short arm of chromosome no 5 giving birth to offspring with the Cri du Chat Syndrome.

This report emphasizes the significance of undertaking chromosome investigation in the parents of all children with chromosome anomalies as it appears that parents such as these may be mosaics to a greater extent than previously presumed. (To be published in *J Med Genet*)

### Discussion

*Lise Wagner* What was the mother's voice like?

*N J Brandt* Normal

*H Dyggve* How many cases are there in Denmark?

*J Philip* This is not known but Niebuhr has hitherto found 20 among mentally retarded patients.

*B Friis Hansen & J Clausen* *Lipid metabolism in mothers and newly born infants*

On account of the increasing difficulty in obtaining breast milk in recent years it has become increasingly necessary to employ breast

milk substitutes and development has tended towards provision of humanized milk. In doing this the number of unsaturated fatty acids has been increased by removing milk fat and replacing this by vegetable oils which are rich in polyunsaturated fatty acids. This together with the interest in the etiology of the degenerative conditions of the central nervous system and the present investigations have together aroused interest in this department in the relationship between the serum lipids in mothers and newly born infants and alterations in the serum lipid levels in infants receiving different diets. The results are expressed as a percentage of the total fatty acid content following fat extraction in milk or serum and gas chromatographic investigation of the fat.

The linoleic acid content of breast milk is approximately 9-12%. In cows milk on the other hand the content is approximately 4% in a half-skimmed milk preparation (Eldon) it is 7% and in a fat substituted milk preparation (Semper) it is about 18-26%. In cord blood the linoleic acid content is approximately 13%. In contrast the content of arachidic acid in maternal blood is 4-5% compared with 8% in cord blood. After the elapse of a week the content of linoleic acid in breast fed infants increases to 10-15% while in infants fed on preparation Semper this increases to the double viz approximately 30%. The importance of the breast milk substitutes containing a well balanced mixture of unsaturated fatty acids is emphasized because too high content of an individual fraction can inhibit the metabolism of the other fractions. Further administration of large quantities of linoleic acid can result in a relative deficiency of vitamin E.

## Discussion

*J Vesterdal* Acidosis has been described in premature infants fed on acidified milk preparations. Feeding with cows' milk has been employed since Auld's time without deficiency symptoms being described.

*N J Brandt* What is the vitamin E content?

*J Clausen* The vitamin E content is 20% lower in skim milk preparations than in the other preparations.

*E Nøthen* Are these quantities in weight per cent?

*J Clausen* The determinations are carried out by gas chromatography and thus express the content by weight.

*J Clausen & B Fris Hansen* *Lipoproteins in newly born infants*

Investigations of lipid metabolism in adults in recent years has revealed that the lipid absorbed from the food after transport to the liver or directly in the intestinal walls is taken up or combined partly in the chylomicron fraction and partly in the lipoprotein fractions which migrate electrophoretically in the  $\alpha$  and  $\beta$  zones (cf. review by Burstein 1961). Further a lipoprotein occurs in serum and does not possess the abovementioned transport function.  $\beta$  lipoprotein consists immunologically of at least two fractions with partial immunological identity (viz.  $\alpha 2$  and  $\beta$  lipoproteins) (3). By means of electrophoretic separation in albumin-containing agarose the  $\beta$  lipoprotein can be separated from the  $\alpha 2$  lipoprotein which is also termed pre  $\beta$  lipoprotein which is of diagnostic significance for subdivision of anomalies of lipoproteins (2).

At birth the newly born organism undergoes transition from parenteral to oral feeding. In agreement with the parenteral feeding of the foetus the authors found on investigation of cord blood that newly born infants lack or have a very low content of  $\beta$  and  $\alpha 2$  lipoprotein, the dominating lipid fraction in cord

blood being a fraction with an electrophoretic migration resembling  $\alpha 1$  lipoprotein.

By means of electrophoresis in albumin-containing agarose it was found as a function of the time which had elapsed since birth that the quantity of  $\beta$  lipoprotein rose in the course of the first two weeks. By means of quantitative radial immunodiffusion it was demonstrated that the adult  $\beta$  lipoprotein level is not attained until the second or third week of life at the earliest and that this is influenced by individual differences.

Continued investigations will reveal whether retarded development of  $\alpha$  and  $\beta$  lipoproteins are connected with the well being of infants.

## REFERENCES

1. Burstein M. *Bull Acad Suisse Sci Med* 17: 92, 1961.
2. Fredrickson D S & Lees R. S. *Circulation* 31: 321, 1965.
3. Lawrence S H & Stein F C. *Science* 137: 227, 1962.

## Discussion

*N J Brandt* Are chylomicrons seen with this technique?

*J Clausen* No.

*J Vesterdal* Is it not possible that transport lipoproteins are present in newly born infants but do not become manifest until lipids are present?

*J Clausen* In hyperlipidaemia the protein fraction varies but not the quantity of lipids which appears to contradict the above pre-assumption.

*N Hobolth* How were these infants fed?

*J Clausen* As a rule with breast milk.

*P Krasilnikoff* Are  $\beta$  and  $\alpha 2$  lipoproteins equally suitable for lipid transport?

*J Clausen* The  $\beta$  lipoproteins are the most important and  $\alpha 2$  lipoproteins only constitute 10 per cent of the total fraction in normal adults.

P Parregaard

## BOOK REVIEWS

V Dubowitz *The floppy infant* Clinics in Developmental Medicine No 31 W Heinemann Medical Books Ltd London 1969 109 pp US \$6.00

On 100 pages Victor Dubowitz reviews the causes and the management of muscular hypotonia in infancy. His description is clear and concise and well illustrated with pictures of children with muscular hypotonia of various causes and with the histopathological findings in the muscles particularly in the rare forms of congenital myopathy.

The author uses the presence or absence of true weakness in the hypotonic infant as the first clue to a differential diagnosis. The conditions characterized by muscular weakness are surveyed in chapter III. The quantitative dominance of progressive spinal muscular atrophy is stressed and the various types of rare and recently described congenital myopathies are well outlined and illustrated with personal cases.

In the next chapter conditions causing muscular hypotonia without weakness are reviewed. The emphasis is here on disorders associated with mental retardation, certainly the most common cause of muscular hypotonia in early infancy. Examinations useful in establishing a differential diagnosis are briefly outlined.

The approach recommended by the author always first to decide whether or not true weakness is present and then proceed along one of 2 different lines is sensible and rewarding in most cases. However the author has not entirely followed his own outline. Thus conditions included in chapter 3 seem to have the anatomical localisation within the motor unit in common rather than the presence of the clinical sign weakness and in chapter 4 conditions with definite weakness but without evidence of involvement of the motor unit are also described. A severe perinatal lesion of the cervical cord must cause both hypotonia and weakness and so does Prader-Willi's syndrome both are described in chapter 4. It is true that the profound hypotonia seen in Prader-Willi's syndrome is out of proportion to the moderate weakness that is however often so severe that these patients are misdiagnosed as cases of muscular dystrophy. A line about Prader-Willi's syndrome in chapter 3 had therefore been helpful to the clinician trying to diagnose a given case of muscular weakness and hypotonia.

In chapter 5 the diagnostic procedures are described and the conditions outlined in which certain procedures are of special help. The great value of a muscular biopsy is stressed somewhat at the expense of electromyography which should have been mentioned as a necessary procedure to establish or ex-

clude myotonic dystrophy in a young infant. The difficulties stressed by the author in interpreting the electromyogram of an infant may decrease with the examiner's increasing experience of the procedure in this age group. It adds greatly to the value of the book that the author also gives a description of when and how to take a muscular biopsy and how to handle it before it reaches the pathologist. As the author recommends the quadriceps muscle for routine biopsy it should perhaps be pointed out that intramuscular injections given in the muscle interfere with the histological picture to at least the same extent as an EMG needle.

Doctor Dubowitz's approach to the problem of infantile muscular hypotonia is sensible and thoroughly enjoyable for the clinician. The patient presents himself to the doctor with a series of symptoms not with a diagnosis and all clinical teaching ought to start with symptoms and symptoms and signs lead up to a diagnosis. This book is obviously written by an excellent clinician and teacher of clinical medicine and it can be highly recommended to all physicians dealing with young children.

Ingrid Gemstorp

H G Evans (ed) *Immunisation against infectious diseases* British Medical Bulletin 25 No 2 London 1969 £2

Several British authorities contribute to the information given in this journal. After an introductory article on world problems in viral vaccines and a survey of the search for protective antigens more specific information is given about the different viral and bacterial vaccines.

Some authors have pointed out that the risks of damaging complications from smallpox vaccination are so great that it should not be justified in countries free from infection. However the importation of smallpox into several countries in Europe recently makes thorough programs of vaccination desirable. Use of antivaccinia gamma globulin is indicated in cases of vaccinia gangrenosa and as a prophylactic measure in higher risk groups. It also seems to be successful in preventing postvaccinal encephalitis.

The choice of killed or attenuated polio vaccine is still open to some controversy. In most European countries and in the United States the attenuated vaccine is the most generally accepted both for routine prophylaxis and epidemic control. However there are disadvantages such as inhibition in the gut

by interfering viruses and risk of causing paralysis. Swedish investigators have shown that killed vaccines produce both short and long term immunity which serologically measured is as good as that produced by attenuated vaccines.

Clinical trials have now been carried out with measles vaccines and approximately 35 million doses of an attenuated vaccine have been used. Reports of severe diseases associated with vaccination have been few. The degree of protectiveness is very high.

Live attenuated rubella vaccines have been administered to several thousand susceptible individuals mainly children. Mild reactions consisting of upper respiratory symptoms, lymphadenopathy and involvement of joints have been reported.

No mention is made of vaccinations against mumps although some clinical trials have already been carried out.

At the moment vaccines against upper respiratory infections make no significant contribution to public health. They had to contain so many components, perhaps more than 100, that it is not surprising that preparation of such vaccines has not yet been reported.

Evidence is now accumulating that BCG is of value not only in protecting against tuberculosis but against other mycobacterial infections as well.

Unwanted reactions to combined vaccines containing pertussis vaccines have been attributed to the pertussis component. It is stated that the reaction rate is significantly lower in a series in which a triple vaccine with an aluminium adjuvant is used. No comments are made on complications from the nervous system.

Concerning typhoid paratyphoid vaccines it is said that certain vaccines containing Salm typhi are effective in the field. The value of the paratyphoid components is doubtful. This is in accordance with clinical experience from many other places.

The journal also contains instructive articles on rubella vaccine, arbovirus vaccines, rickettsial vaccines, diphtheria and tetanus toxoids, cholera vaccines, prophylactic vaccines and the use of known immunoglobulins. It is strongly recommended to all those interested in the prophylaxis of infectious diseases and in public health.

Ulf Bergdahl

D J Baetema. *A neurological study of newborn in Janus Clinics in developmental medicine*. No 28. W Heinemann Medical Books Ltd, London 1968. 178 pp. illus 37.

In sample 49 full term infants neurological examinations were made every day for the first 9 days after birth. Infants with severe abnormalities or who were ill were eliminated from the study. The neurological examination used is the one previously developed in Groningen (Precht & Baetema 1964). Each item was scored on a quantitative scale and all data were statistically analysed. The purpose of this

investigation was to study the consistencies and in consistencies of the neonatal neurological signs. Three basic questions were asked.

1 *What standardisation procedures should the examiner introduce in order to get reliable information from the neurological examination?* Ambient temperature, clothing and hunger which influence the nervous function of newborn infants are very well controlled in this study. The state (of sleep and wakefulness) of the infant was studied before and during the neurological examination. The time course and alterability of the state by handling the baby were investigated. One of the conclusions is that the optimal state for many of the neurological tests is less often present during the first days after birth.

2 *What is the developmental course of neurological signs during the first days of life?* The data regarding age dependence showed three kinds of development of the neurological signs: (a) signs which were weaker or more difficult to elicit on the 1st-4th days than on the 5th-9th (e.g. responses in which motility is involved); (b) a small group opposite to (a) i.e. signs which were weaker on the 5th-9th days than on the 1st-4th (e.g. resistance to passive movements of the forearm and elbow); (c) signs which remain constant throughout the neonatal period (e.g. abdominal skin reflexes, blink reflexes, tendon reflexes). Another important point is the persistence of a characteristic behavior pattern. It was concluded that from about the 4th day the findings were representative for the rest of the neonatal period which means that a neurological examination carried out on the first three days is less valid than those in later days.

3 *Do obstetrical and perinatal conditions affect the developmental course and the consistency of neurological signs throughout the neonatal period?* Pre- and perinatal obstetrical data were compared with the daily scores of neurological items. In addition a high risk and a low risk group were compared. Infants with the umbilical cord around the neck and apnoea at birth often got an apnoeic syndrome on the first 4 days. The difference found between the low and high risk groups was that babies of the latter group had lower resistance to passive movements on the first day and lower in tension for some other tests. It was concluded that infants who had suffered from perinatal complications often had a prolonged adaptation period—a general depression of the nervous system on the first few days. Most of the infants who had a moderate resistance to passive movements on the first day and became hypotonic later on appeared to be jaundiced and/or had gastro-intestinal signs later.

Prof. Heinz Precht points out in the preface of this book that the knowledge of the CNS of the neonate is still scanty. The reader will agree that Baetema's study of the neurology of newborn infants is an excellent attempt to fill the gap. The results of this thorough, critical and interesting study which have been presented as a thesis are of great importance for the evaluation of neurological examinations.



tions of neonates. Thus the book can be warmly recommended to all paediatricians or neurologists concerned with such examinations.

*Jan Ekholm*

J. G. Howells (ed) *Modern perspectives in international child psychiatry*. Oliver & Boyd, Edinburgh, 1969. 878 pp. £8.8s.

Perhaps child psychiatry is the speciality that has had most difficulties in finding its identity among medical professions. In his introduction to *Modern Perspectives in International Child Psychiatry*, Leo Kanner says: 'In the light of present-day usage it sounds incredible that the term child psychiatry itself had not acquired formal citizenship in the realm of professional or any other parlance until a little less than three decades ago. The reason for this is that child psychiatry, more than other medical disciplines, is founded on the behavioural sciences. Child psychiatry is a fusion of what used to be a collection of more or less loosely scattered segments. Its roots are not only in psychiatry proper and in pediatrics but also in education, psychology, criminology and sociology. For the medical student with his traditional training in the natural sciences, there may be a certain difficulty to find his professional ego in a discipline where so many non-medical sciences also

dominate the scene. But on the other hand, even if it is difficult to define child psychiatry as a medical profession, it surely exists as a functional concept and as a result of many pressing needs to solve mental problems in childhood.'

This new edition of *Modern Perspectives* may be seen as an illustration of Kanner's notion that child psychiatry is a result of the convergence of many interests. The authors represent very different approaches and professions. Contributions come from Canada, France, Germany, Israel, Italy, Japan, Sweden, The United Kingdom, The United States, and The USSR. This work ought to be of great interest not only to child psychiatrists but also to those working in related professions and specialties—medical or non-medical. There are twenty-six different articles treating subjects such as adverse perinatal factors (Stolt), separation and deprivation (Howells), the management of ulcerative colitis (Prush & Jordan), childhood psychosis (Bender), residential care of children (Ahmjo) and the strategy of follow-up studies (Robins & O'Neal) to take only a few. Together with the first edition of *Modern Perspectives in Child Psychiatry* (2nd ed. 1967) this book is a very valuable source of knowledge in child psychiatry and perhaps the most comprehensive available at present. It surely gives the impression that child psychiatry has an identity of its own.

*Michael Bohman*

## ANNOUNCEMENT

Under the auspices of the International Pediatric Association, the Iranian Society of Pediatrics in collaboration with the Middle Eastern Mediterranean Pediatric Society is organizing in Tehran from Sept.

13-16 1970 the VII Middle Eastern Mediterranean Pediatric Congress. For additional information, please write Iranian Society of Pediatrics, P.O. Box 14/1761, Tehran, Iran.

## INTENSIVE CARE OF SMALL PREMATURE INFANTS

## I. Clinical findings and results of treatment

EERO K. VAPAAJÄRVI and NIELS C. R. RÄTHJÄ

From the Department of Pediatrics, University Central Hospital, Helsinki, Finland

During recent years there has been a growing concern over the intensive care of newborn infants suffering from respiratory insufficiency. Most reports, however, include only a few cases of small premature infants (1-8, 23, 24) who are a high risk group with high mortality and high incidence of respiratory failure requiring assisted ventilation. On the other hand, follow-up studies reported by a number of workers (8, 10, 13) indicate that the incidence of severe brain damage in these infants is very high. The deficiency of detailed clinical data during the neonatal period, however, make it difficult to draw any conclusions about the possible etiologic factors responsible for the neurologic and mental abnormalities indicative of permanent brain injury.

Thus, we found it important to conduct a prospective study on the intensive care of premature infants with birth weights between 850 and 1250 g, and to discuss the correlation between clinical findings, response to the treatment and outcome.

## MATERIAL AND METHODS

The study comprised 49 premature infants weighing from 850 to 1250 g at birth, admitted to the Neonatal Unit of the University Children's Hospital, Helsinki, during the two-year period 1966-1967. All infants with major congenital malformations were excluded.

Supported in part by grants from the Foundation for Pediatric Research in Finland and the Association for the Aid of Crippled Children, New York.

All infants were nursed in incubators with a high environmental humidity and maintained skin temperature at 36.5°C. As soon as possible after admission, one of the umbilical arteries was catheterized and following a 10 minute period of breathing 90-100% oxygen arterial pH,  $P_{CO_2}$  and  $P_{O_2}$  were determined (19). The umbilical vein was also catheterized to begin intravenous early feeding using 10 g/lucose solution (50 ml per kg per 24 hrs). To prevent infection, antibiotics, usually cloxacillin (50 mg per kg per 24 hrs) and colistymycin (30 000 units per kg per 24 hrs) were given intramuscularly in two divided doses. To correct acidosis, rapid sodium bicarbonate infusions were given according to the acid base status. A chest roentgenogram was obtained if possible during the first 24 hours of life.

Apnea, which could not be overcome by peripheral stimulation, was treated with endotracheal intubation and short (manual) ventilation. Accumulation of  $CO_2$  (over 80 mm of mercury) and falling  $P_{O_2}$  in arterial blood (below 50) were also indications for starting intermittent positive pressure respiration (IPPR). A positive pressure respirator (BENNETT PR 1 or PR 2) and occasionally manual ventilation was used. The inspired oxygen concentration was adjusted to maintain the arterial  $P_{O_2}$  between 50 and 100 mm of mercury.

## CLINICAL DEFINITIONS

**Respiratory distress (RD).** All patients were divided into three categories according to the severity of their respiratory symptoms during the first 12 to 4 hours of life. On admission a careful physical examination was made including an evaluation of the following symptoms: 1) cyanosis, 2) expiratory grunting, 3) intercostal and subcostal retractions, 4) abnormal respiratory rate and 5) apneic attacks. Severe birth asphyxia (5 minute Apgar score 0-5) associated with apnea which did not respond to resuscitation or a rapidly progressing respiratory insufficiency including all the symptoms indicated above was defined as severe respiratory distress. The occur-

tions of neonates. Thus the book can be warmly recommended to all paediatricians or neurologists concerned with such examinations.

Jan Ekholm

J G Howells (ed) *Modern perspectives in international child psychiatry*. Oliver & Boyd, Edinburgh, 1969. 878 pp. £8.85.

Perhaps child psychiatry is the speciality that has had most difficulties in finding its identity among medical professions. In his introduction to *Modern Perspectives in International Child Psychiatry*, Leo Kanner says: "In the light of present-day usage it sounds incredible that the term child psychiatry itself had not acquired formal citizenship in the realm of professional or any other parlance until a little less than three decades ago. The reason for this is that child psychiatry more than other medical disciplines is founded on the behavioural sciences. Child psychiatry is a fusion of what used to be a collection of more or less loosely scattered segments." Its roots are not only in psychiatry proper and in pediatrics but also in education, psychology, criminology and sociology. For the medical student with his traditional training in the natural sciences there may be a certain difficulty to find his professional ego in a discipline where so many non-medical sciences also

dominate the scene. But on the other hand, even if it is difficult to define child psychiatry as a medical profession, it surely exists as a functional concept and as a result of many pressing needs to solve mental problems in childhood.

This new edition of *Modern Perspectives* may be seen as an illustration of Kanner's notion that child psychiatry is a result of the convergence of many interests. The authors represent very different approaches and professions. Contributions come from Canada, France, Germany, Israel, Italy, Japan, Sweden, The United Kingdom, The United States and The USSR. This work ought to be of great interest not only to child psychiatrists but also to those working in related professions and specialties—medical or non-medical. There are twenty-six different articles treating subjects such as adverse perinatal factors (Stott), separation and deprivation (Howells), the management of ulcerative colitis (Prugh & Jordan), childhood psychosis (Bender), residential care of children (Ahmaji) and the strategy of follow-up studies (Robins & O'Neal) to take only a few. Together with the first edition of "Modern Perspectives in Child Psychiatry" (2nd ed. 1967) this book is a very valuable source of knowledge in child psychiatry and perhaps the most comprehensive available at present. It surely gives the impression that child psychiatry has an identity of its own.

Michael Bohman

## ANNOUNCEMENT

Under the auspices of the International Pediatric Association, the Iranian Society of Pediatrics in collaboration with the Middle Eastern Mediterranean Pediatric Society is organizing in Tehran from Sept

13-16 1970 the VII Middle Eastern Mediterranean Pediatric Congress. For additional information please write Iranian Society of Pediatrics P.O. Box 14/1761 Tehran, Iran.

## INTENSIVE CARE OF SMALL PREMATURE INFANTS

## I Clinical findings and results of treatment

EERO A. VAPAAVUORI and NIELS C. R. KAHIA

From the Department of Paediatrics, University Central Hospital, Helsinki, Finland

During recent years there has been a growing concern over the intensive care of newborn infants suffering from respiratory insufficiency. Most reports, however, include only a few cases of small premature infants (1, 6, 23, 24) who are a high risk group with high mortality and high incidence of respiratory failure requiring assisted ventilation. On the other hand, follow-up studies reported by a number of workers (8, 10, 13) indicate that the incidence of severe brain damage in these infants is very high. The deficiency of detailed clinical data during the neonatal period, however, make it difficult to draw any conclusions about the possible etiologic factors responsible for the neurologic and mental abnormalities indicative of permanent brain injury.

Thus, we found it important to conduct a prospective study on the intensive care of premature infants with birth weights between 850 and 1250 g, and to discuss the correlation between clinical findings, response to the treatment and outcome.

## MATERIAL AND METHODS

The study comprises 49 premature infants weighing from 850 to 1250 g at birth, admitted to the Neonatal Unit of the University Children's Hospital, Helsinki, during the two-year period 1966-1967. All cases with major congenital malformations were excluded.

Supported in part by grants from the Foundation for Pediatric Research in Finland and the Association for the Aid of Crippled Children, New York.

All infants were nursed in incubators with a high environmental humidity and maintaining skin temperature at 36.5°C. As soon as possible after admission, one of the umbilical arteries was catheterized and following a 10 minute period of breathing 90-100% oxygen, arterial pH,  $P_{CO_2}$  and  $P_{O_2}$  were determined (19). The umbilical vein was also catheterized to begin intravenous early feeding using 10 g/lucose solution (30 ml per kg per 24 hrs). To prevent infection, antibiotics, usually cloxacillin (50 mg per kg per 24 hrs) and colistin (30 000 units per kg per 24 hrs) were given intramuscularly in two divided doses. To correct acidosis, rapid sodium bicarbonate infusions were given according to the acid base status. A chest roentgenogram was obtained if possible during the first 24 hours of life.

Apnea which could not be overcome by peripheral stimulation was treated with endotracheal intubation and short (manual) ventilation. Accumulation of  $CO_2$  (over 60 mm of mercury) and falling  $P_{O_2}$  in arterial blood (below 50) were also indications for starting intermittent positive pressure respiration (IPPR). A positive pressure respirator (BEN-VEET PR I or PR 2) and occasionally manual ventilation was used. The inspired oxygen concentration was adjusted to maintain the arterial  $P_{O_2}$  between 50 and 100 mm of mercury.

## CLINICAL DEFINITIONS

*Respiratory distress (RD).* All patients were divided into three categories according to the severity of their respiratory symptoms during the first 12 to 24 hours of life. On admission, a careful physical examination was made including an evaluation of the following symptoms: 1) cyanosis, 2) expiratory grunting, 3) intercostal and subcostal retractions, 4) abnormal respiratory rate and 5) apneic attacks. Severe birth asphyxia (5 minute Apgar score 0-3) as associated with apnea which did not respond to resuscitation or a rapidly progressing respiratory insufficiency including all the symptoms indicated above was defined as severe respiratory distress. The occur-

tions of neonates. Thus the book can be warmly recommended to all paediatricians or neurologists concerned with such examinations.

Jan Ekholm

J G Howells (ed) *Modern perspectives in international child psychiatry*. Oliver & Boyd, Edinburgh, 1969. 878 pp. £8.8s.

Perhaps child psychiatry is the speciality that has had most difficulties in finding its identity among medical professions. In his introduction to *Modern Perspectives in International Child Psychiatry*, Leo Kanner says: 'In the light of present-day usage it sounds incredible that the term child psychiatry itself had not acquired formal citizenship in the realm of professional or any other parlance until a little less than three decades ago. The reason for this is that child psychiatry more than other medical disciplines is founded on the behavioural sciences. Child psychiatry is a fusion of what used to be a collection of more or less loosely scattered segments. Its roots are not only in psychiatry proper and in pediatrics but also in education, psychology, criminology and sociology. For the medical student with his traditional training in the natural sciences there may be a certain difficulty to find his professional ego in a discipline where so many non-medical sciences also

dominate the scene. But on the other hand even if it is difficult to define child psychiatry as a medical profession it surely exists as a functional concept and as a result of many pressing needs to solve mental problems in childhood.'

This new edition of *Modern Perspectives* may be seen as an illustration of Kanner's notion that child psychiatry is a result of the convergence of many interests. The authors represent very different approaches and professions. Contributions come from Canada, France, Germany, Israel, Italy, Japan, Sweden, The United Kingdom, The United States and The USSR. This work ought to be of great interest not only to child psychiatrists but also to those working in related professions and specialities—medical or non-medical. There are twenty-six different articles treating subjects such as adverse perinatal factors (Stolt), separation and deprivation (Howells), the management of ulcerative colitis (Prugh & Jordan), childhood psychosis (Bender), residential care of children (Ahrensjo) and the strategy of follow-up studies (Robins & O'Neal) to take only a few. Together with the first edition of *Modern Perspectives in Child Psychiatry* (2nd ed. 1967) this book is a very valuable source of knowledge in child psychiatry and perhaps the most comprehensive available at present. It surely gives the impression that child psychiatry has an identity of its own.

Michael Bohman

## ANNOUNCEMENT

Under the auspices of the International Pediatric Association, the Iranian Society of Pediatrics in collaboration with the Middle Eastern Mediterranean Pediatric Society is organizing in Tehran from Sept

13-16, 1970, the VII Middle Eastern Mediterranean Pediatric Congress. For additional information, please write Iranian Society of Pediatrics, P.O. Box 14/1761, Tehran, Iran.

diffuse reticulo granular pattern characteristic of a moderate alveolar respiratory distress syndrome (ARDS) and grade III (Fig. 1c) represents the X ray seen in the most severe cases of IRDS where the configuration of the heart cannot be distinguished from the totally opaque and airless lung fields. The air filled bronchial tree is visualized as the typical air bronchogram. Aspiration and pneumonia can usually be differentiated from the pulmonary changes indicative of IRDS by the more patchy and lobar infiltrations (Fig. 1d).

#### Diagnosis

The clinical diagnosis of IRDS was based on the characteristic respiratory symptoms (expiratory grunting, retraction, cyanosis and high respiratory rate) and on the chest X ray findings. In most cases of suspected intrauterine pneumonia there was a history of early rupture of the foetal membranes associated with foetal smearing amniotic fluid at birth. In addition chest X rays revealed patchy and lobar infiltrations in some of the cases.

A diagnosis of massive intraventricular brain haemorrhage (IVH) was based on a typical course of the illness. Following a comparatively symptomless period sudden collapse with respiratory and circulatory failure was seen occasionally associated with convulsions and a bulging fontanel. Further evidence suggesting IVH was a fall in blood haemoglobin values. In one case only was it possible to confirm the diagnosis by demonstrating blood in the spinal fluid.

## RESULTS

#### Clinical findings

The material is summarized in Table 1. Of the total of 49 infants there were 27 deaths giving an overall mortality rate of 55%. There was no difference between mean birth weights whereas gestational ages show a difference of 2 weeks between survivors and deaths. Fig. 2 illustrates the correlation between birth weight/gestational age and mortality on the intrauterine growth chart by Backstrom & Hansson 1968 (3). Of the infants with gestational ages below 29 weeks 20 died as compared with only 3 dying of the 12 small for date infants (gestational ages over 30 weeks).

Detailed clinical data for the survivors are presented in Table 2. There was only one surviving case defined as belonging to the group of severe RD. This infant survived prolonged IPPR begun due to severe IRDS. In the group of moderate RD there were 6 cases diagnosed

Table 1 Summary of the material

Data	Survivors	Deaths
Total number of infants	22	27
Mean birth weight (g)	1090	1090
(range)	(900-1250)	(850-1230)
Mean gest. age (weeks)	29	27
(range)	(24-34)	(24-34)
Complications during pregnancy and delivery	15	22
Sex M/F	12/10	15/12

as having IRDS of whom 4 showed only transient signs indicating pulmonary hyaline membranes. There were 3 cases suffering from apnoeic attacks which could not be overcome by short resuscitation and where IPPR was started at the age of 21, 78 and 96 hours respectively. No signs indicating IRDS were seen in these cases. Of the remaining 5 infants 2 had hypoglycaemia and 3 were symptomless. Of the 7 cases suffering from slight or no RD one was treated on IPPR for an apnoeic attack occurring at the age of 72 hours. There were 2 small for dates infants of toxemic mothers and the remaining 4 were symptomless.

Table 3 presents the clinical data for the expired cases treated on IPPR and grouped according to the survivors into 3 categories of

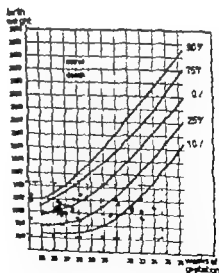


Fig. 2 Correlation between birth weight/gestational age and mortality on the growth chart by Backstrom & Hansson 1968 (3).

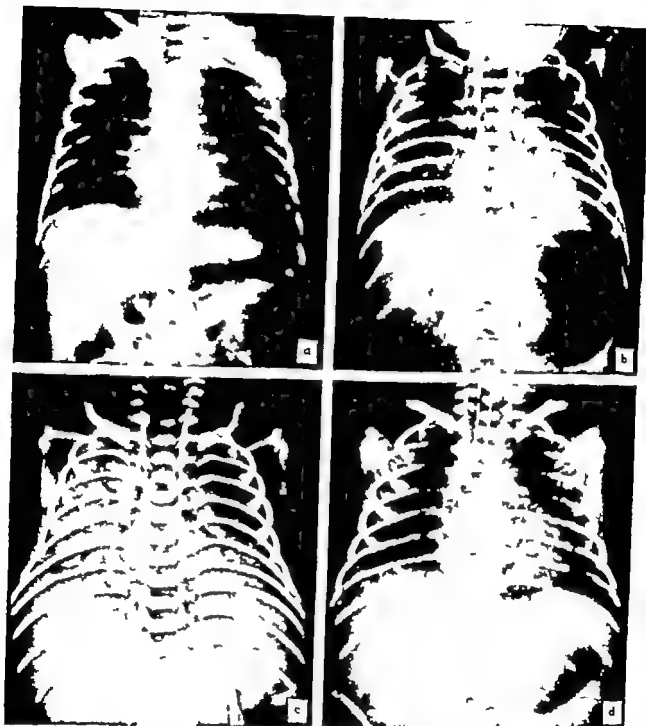


Fig 1 Chest roentgenograms grade I to III (a-c) illustrating the reticulo granular density and air

bronchogram characteristic of IRDS and patchy alveolar infiltrations indicating pneumonia (d)

rence of some of the respiratory symptoms following slight or moderate birth asphyxia (5 minute Apgar score 4-7) and a more gradual progression of the respiratory symptoms was termed *moderate respiratory distress*. Finally when there was usually no birth asphyxia (5 minute Apgar score 7 or more) and only transient or mild respiratory symptoms occasionally followed by an *unexpected apnoeic attack requiring mechanical ventilation* the condition was defined as *slight or no respiratory distress*. It must be emphas-

ized however that in this special weight group some degree of retraction and irregular breathing is often seen in otherwise symptomless surviving infants.

#### Chest roentgenograms

The chest roentgenograms have been divided into three categories according to the degree of reticular granularity as illustrated in Fig 1 grade I (Fig 1a) shows only slightly increased densities and also included normal findings grade II (Fig 1b) shows the

Table 3 Deaths detailed clinical data grouped according to the three categories of respiratory distress

(IRDS = idiopathic respiratory distress syndrome IVH = intraventricular brain hemorrhage INF = infection PH = massive pulmonary hemorrhage BPD = bronchopulmonary dysplasia)

Case no	B W (g)	Gest age	Complic factors at birth	Birth asphyxia	Age on admission hr/min	Onset of IPPR (hr)	Chest X ray	Init art		Diagnosis
								pH	P mm Hg	
I Severe RD										
23	1100	34	Cesarean section	Severe	—/35	At birth	II	7.07	30	IRDS PH
24	870	28	Ablatio plac	Severe	1/—		II	6.99	—	IRDS
25	1000	26	Hydranion	Severe	1/30		III	7.25	40	IRDS PH
26	1100	26	Early rupture of the membranes	Severe	—/30		—	6.55	33	INF IVH
27	1080	28	—	Severe	4/5	6	I	7.09	—	IVH
28	1030	6	Placenta previa	Moderate	5/—		III	7.06	39	IRDS
29	1250	24	Twin birth B	Moderate	4/—		III	7.17	78	IRDS
30	1230	28	Twin birth B	No	3/40		III	7.25	—	IRDS
31	880	5	Early rupture of the membranes	Moderate	3/30	6	II	6.85	24	INF
32	1270	31	Twin birth A	No	2/—	14	III	7.06	80	IRDS BPD
33	1000	28	Early rupture of the membranes	No	—/40	1	I	6.96	26	IVH PH
II Moderate RD										
34	1200	28	Hydranion	No	1/—	15	—	7.15	—	IRDS
35	850	77	Twin birth A	No	3/45	125	I	7.12	41	IVH pneumoth
36	1230	33	Breech delivery	Severe	2/45	23	—	7.31	470	IVH INF
37	1100	27	Early rupture of the membranes	No	7/10	29	II	7.07	64	INF IRDS
38	860	24	—	No	1/35	3	I	6.90	124	IRDS
39	1000	26	Cesarean section	Moderate	1/45	5	II	7.22	8	IRDS PH
40	990	—	Twin birth B	Moderate	3/18	111	I	7.21	260	IRDS IVH
III Slight or no RD										
41	1100	27	—	No	9/30	96	II	7.26	—	Apnea pneumoth
42	1100	9	—	No	1/45	96	II	7.28	360	IVH pneumoth
43	1040	26	Early rupture of the membranes	Moderate	3/—	6	I	7.04	400	IRDS INF
44	940	26	Cesarean section	No	—/20	15	I	7.31	250	Apnea INF (sec)
45	1110	7	Early rupture of the membranes	No	—/30	9	I	7.32	475	INF IVH
46	1000	25	Early rupture of the membranes	No	46/—	59	I	7.29	430	INF
47	1000	27	—	No	—/10	15	I	7.18	510	Apnea INF (sec)
48	1100	—	Early rupture of the membranes	Moderate	2/—	15	I	7.22	85	INF IRDS
49	990	7	Breech delivery	Moderate	1/5	28	—	7.2	—	IVH

In Table 5 the mean values of initial arterial pH  $P_{CO}$  and  $P_{O_2}$  (determined following a 10 minute period of breathing 90–100% oxygen) and the calculated H L shunt percentages (16) are given. The most marked differences are seen in pH  $P_{O_2}$  and R L shunt values in the expired cases of severe RD. pH values below 7.00 were found in 4 of 5  $P_{O_2}$  values were below 100 mm of mercury and the R L shunt percentage (mean) was 50% as compared with

28% in survivors. Both pH and  $P_{O_2}$  were remarkably higher in the other two groups and there was also a striking difference between survivors and deaths. As an interesting finding there were only 3 surviving infants with initial pH values below 7.20.

#### Response to IPPR

In Fig. 3 the response to mechanical ventilation as demonstrated by changes in arterial



Table 2 *Survivors detailed clinical data grouped according to the three categories of respiratory distress*

(IRDS = idiopathic respiratory distress syndrome IVH = intraventricular brain hemorrhage INF = infection PH = massive pulmonary hemorrhage BPD = bronchopulmonary dysplasia RLF = retrolental fibroplasia)

Case no	B W (g)	Gest age	Complic factors at birth	Birth asphyxia	Age on admission hr/min	Onset of IPPR (hr)	Chest X ray	Int art		Diagnosis
								pH	P mm Hg	
I Severe RD										
1	1160	31	Hydramnion breech delivery	Moderate	1/—	32	II	7.21	140	IRDS
II Moderate RD										
2	1250	28	Breech delivery	Severe	1/15	—	I	7.26	195	IRDS
3	995	27	Caes section	No	—/30	—	—	7.15	—	IRDS
4	1200	28	—	No	3/5	—	I	7.14	—	IRDS (transient)
5	1705	30	Twin birth A	Moderate	1/30	—	I	7.25	164	IRDS (transient)
6	1000	26	Hydramnion	Severe	8/30	—	I	7.30	345	IRDS (transient)
7	920	33*	Twin birth A	Moderate	2/—	—	I	7.28	220	IRDS (transient)
8	1020	33*	Twin birth II	Moderate	2/—	—	I	7.35	480	Symptomless
9	1090	27	—	Moderate	2/10	—	—	7.25	—	Symptomless
10	1160	29	Twin birth B	No	2/5	—	I	7.21	—	Hypoglycemia
11	1040	32	Caes section	No	—/37	—	I	7.22	470	Hypoglycemia
12	960	31	Twin birth B	?	2/30	—	I	7.24	440	Symptomless
13	980	26	Breech delivery	Moderate	8/—	96	I	7.35	335	Apnea RLF
14	1120	27	—	No	14/—	21	I	7.14	340	Apnea BPD
15	1250	24*	—	No	6/25	78	I	7.19	215	Apnea IRDS (transient)
III Slight or no RD										
16	1150	32*	—	No	7/30	72	I	7.32	420	Apnea
17	1170	34	Maternal bleeding	No	—/40	—	—	7.32	435	Symptomless
18	1100	32	Breech delivery	No	—/50	—	I	7.22	420	Hypoglycemia
19	1050	28	—	No	11/30	—	I	7.32	290	Symptomless
20	1150	24	—	Moderate	2/45	—	I	7.25	290	Symptomless
21	1100	28	Ablatio plac	No	1/10	—	—	7.22	—	Symptomless
22	900	28	Twin birth B	No	19/5	—	I	7.34	490	Symptomless

RD The postmortem findings are discussed in detail in the second part of the study (26)

From Tables 2 and 3 it is clear that severe RD is almost invariably fatal (only one survivor). The majority of cases having moderate RD survived (14 of 21) whereas there were more deaths (9 of 16) in the category of slight or no RD. This is caused by the occurrence of unexpected respiratory failure following a comparatively symptomless period in the fatal cases of intrauterine infection (4 cases) and intraventricular brain hemorrhage (2 cases).

As demonstrated in Table 4 the incidence of birth complications is markedly higher in category I (92%) as compared with only 76% and 63% in categories II and III respectively. As could be expected there were more moderately or severely asphyxiated infants in cate-

gories I and II. All cases of severe RD were treated on IPPR whereas 10 of 21 and 10 of 16 respectively required assisted ventilation in categories II and III.

As also demonstrated in Tables 2 and 3 there was a good correlation between the degree of reticulo granular density in chest X rays and severe IRDS. 9 of the 11 chest X rays revealed changes of grade II and III. Of the surviving cases however only the case of severe IRDS also requiring IPPR showed the grade II changes. In the cases of intrauterine infection the first chest X rays (obtained during the first 24 hours after birth) were considered normal in all but two cases and the patchy and lobar infiltrations indicating pneumonia were seen only during the later course of the illness.

Table 3 Deaths: detailed clinical data grouped according to the three categories of respiratory distress

(IRDS = idiopathic respiratory distress syndrome; IVH = intraventricular brain haemorrhage; INF = infection; PH = massive pulmonary haemorrhage; BPD = bronchopulmonary dysplasia)

Case no.	B.W. (g)	Gest. age	Complic. factors at birth	Birth asphyxia	Age on admission hr/min	Onset of SPPH (hr)	Chest X ray	Init. art.		Diagnosis
								pH	P <sub>a</sub> , mm Hg	
I Severe RD										
23	1100	34	Caes section	Severe	—/35	At birth	II	7.07	30	IRDS PH
24	870	28	Abnormal plac.	Severe	1/—		II	6.99	—	IRDS
25	1000	26	Hydranion	Severe	1/30		III	7.25	40	IRDS PH
6	1170	26	Early rupture of the membranes	Severe	—/20		—	6.53	33	INF IVH
27	1080	28	—	Severe	2/45	6	I	7.09	—	IVH
28	1090	26	Placenta previa	Moderate	5/—		III	7.06	59	IRDS
9	1210	24	Twin birth B	Moderate	4/—		III	7.17	78	IRDS
30	1230	28	Twin birth B	No	3/40		33	7.23	—	IRDS
31	880	25	Early rupture of the membranes	Moderate	5/30	6	II	6.85	21	INF
32	1100	31	Twin birth A	No	2/—	14	III	7.06	80	IRDS BPD
33	1000	28	Early rupture of the membranes	No	—/40	1	I	6.96	26	IVH PH
II Moderate RD										
34	1200	28	Hydranion	No	1/—	13	—	7.1	—	IRDS
35	850	27	Twin birth A	No	3/45	123	I	7.12	41	IVH pneumoth
36	1230	33	Breach delivery	Severe	2/45	—	—	7.11	470	IVH INF
37	1100	27	Early rupture of the membranes	No	7/10	28	II	7.07	66	INF IRDS
38	960	24	—	No	1/35	3	I	6.90	174	IRDS
39	1000	26	Caes section	Moderate	1/45	5	II	7.22	8	IRDS PH
40	990	27	Twin birth B	Moderate	3/18	111	I	7.21	260	IRDS IVH
III Single or no RD										
41	1100	27	—	No	9/30	96	II	7.26	—	Apnea pneumoth
42	1100	—	—	No	1/45	96	II	7.28	360	IVH pneumoth
43	1050	6	Early rupture of the membranes	Moderate	3/—	6	I	7.04	200	IRDS INF
44	940	26	Caes section	No	—/20	15	I	7.31	280	Apnea INF (sec)
45	1110	—	Early rupture of the membranes	No	2/30	93	I	7.22	415	INF IVH
46	1000	25	Early rupture of the membranes	No	46/—	59	I	7.29	230	INF
47	1000	27	—	No	—/30	55	I	7.18	510	Apnea INF (sec)
48	1100	—	Early rupture of the membranes	Moderate	2/—	15	I	7.22	35	INF IRDS
49	890	—	Breach delivery	Moderate	1/25	28	—	7.22	—	IVH

In Table 5 the mean values of initial arterial pH,  $P_{co}$  and  $P_o$  (determined following a 10-minute period of breathing 90–100% oxygen) and the calculated R:L shunt percentages (16) are given. The most marked differences are seen in pH,  $P_o$  and R:L shunt values in the expired cases of severe RD: pH values below 7.00 were found in 4, all  $P_o$  values were below 100 mm of mercury and the R:L shunt percentage (mean) was 90% as compared with

28% in survivors. Both pH and  $P_o$  were remarkably higher in the other two groups and there was also a striking difference between survivors and deaths. As an interesting finding there were only 3 surviving infants with initial pH values below 7.20.

#### Response to IPIR

In Fig. 3 the response to mechanical ventilation as demonstrated by changes in arterial

Table 4 Complicating factors at birth, birth asphyxia and number of cases treated on IPPR in the 3 categories of respiratory distress (RD)

	Severe RD	Moderate RD	Slight or no RD
Total number of infants	12	21	16
Complicating factors at birth	11 (92 %)	16 (76 %)	10 (63 %)
Birth asphyxia (5 minute Apgar score)			
Severe (0-3)	5	3	—
Moderate (4-7)	4	7	3
No (8-10)	3	11	13
Number of cases treated on IPPR	12	10	10

$P_o$ ,  $P_{co_2}$  and pH is graphically illustrated in four typical cases of apnea, IRDS, massive IVH and infection (pneumonia). It is clearly seen that the initial response to respirator treatment as far as  $P_o$  values are concerned is good in apnea and IVH whereas there is a moderate rise in the case of pneumonia but no rise in IRDS. In contrast to the changes in  $P_o$  there are no corresponding differences between the four cases as demonstrated by rising pH and falling  $P_{co_2}$  values. The cases of apnea and IVH however seem to become hyperventilated as shown by low  $P_{co_2}$  values (around 20 mm of Hg) and high pH values respectively.

#### Results of treatment

Table 6 shows the mortality figures for each major cause of respiratory failure. There were

Table 6 Results of treatment, clinical/autopsy findings and outcome

Clinical/Autopsy findings	Survivors	Deaths	Total
IRDS/HMD	7	12	19
Infection	—	8	8
Apnea	4	2	6
Massive IVH	—	4	4
Pulmonary haemorrhage	—	1	1
Symptomatic	11	—	11

a total of 19 infants diagnosed as having IRDS of the 13 cases treated on IPPR only one survived. Cases of intrauterine infection were invariably fatal as well as cases of massive IVH and of massive pulmonary haemorrhage. Of the 6 cases treated on IPPR for apnea unrelated to IRDS or infection 4 survived and the two deaths followed complications of the treatment: one died of pneumothorax and one of overwhelming infection. There were 11 surviving cases, where no signs indicating pulmonary or extrapulmonary pathology could be demonstrated although some of these infants showed transient signs of moderate RD (5 cases). Arterial blood gases and pH were normal in these cases and the chest X-rays were also considered normal.

All survivors have been followed and the physical examination has been performed at the ages of 3, 6, 12 and 24 months. The follow up study is still in progress. There was one case of retrolental fibroplasia (case no. 13 in Table 2) occurring in the first case of the present series.

Table 5 Initial values of pH,  $P_{co_2}$  and  $P$  after a 10 minute period of breathing 90-100 % oxygen

Data	Severe RD		Moderate RD		Slight or no RD	
	Surv	Deaths	Surv	Deaths	Surv	Deaths
Number of cases	1	11	14	7	7	9
Mean pH	7.21	7.03	7.25	7.14	7.28	7.4
(range)		(6.55-7.25)	(7.14-7.35)	(6.90-7.22)	(7.2-7.34)	(7.04-7.32)
Mean $P_{co_2}$ mm Hg	53	76	53	70	44	62
(range)		(45-150)	(31-82)	(43-130)	(31-58)	(34-140)
Mean $P_o$ mm Hg	140	46	320	153	391	297
(range)		(24-80)	(164-480)	(8-40)	(790-190)	(55-510)
Mean R L shunt	28	50	20	27	17	4
(range)		(41-80)	(11-31)	(19-65)	(14-26)	(1-56)

rated on IPPR who required respirator treatment for nearly four weeks. Only during the first of these weeks was it possible to obtain arterial samples. Thus the  $P_{50}$  values possibly responsible for the development of the retrolental fibroplasia are unknown. There was one case demonstrating the typical symptom-complex of the bronchopulmonary dysplasia (17) (case no. 14 (BPD) in Table 2). Case no. 6 showing signs of severe brain damage at the age of one year was found by pneumo-encephalography to have a brain malformation (single ventricle associated with cortical atrophy). The number of cases showing signs of slight or moderate brain injury is small as compared with the majority of cases where no neurologic abnormalities can be demonstrated.

## DISCUSSION

It is well known that there is a better correlation between gestational age and perinatal mortality than between birth weight and perinatal mortality. As illustrated in Fig. 2 there was a much higher mortality rate in the low gestational age group (24 to 28 weeks) than in the small for date infants (29 to 34 weeks of gestation). Although the incidence of IRDS in different weight groups has been reported by several workers (2, 14, 15, 20) the correlation of the incidence of IRDS with gestational age and birth weight has only infrequently been given in infants weighing less than 1250 g at birth (9). In the present series there is a strikingly higher incidence of severe IRDS requiring IPPR in premature infants with gestational ages below 29 weeks (10 cases of 23) than in infants with gestational ages over 29 weeks (3 of 23).

### *Etiologic factors*

The causal relationship between complications during the delivery leading to intrauterine and postpartum hypoxia and the incidence of hyaline membrane disease is well documented (14, 16). As demonstrated in Tables 2 and 3 there were only a few cases of spontaneous meconium-stained deliveries in the present series. In all but

one of the 12 cases of severe RD the delivery was complicated as compared with 6 uneventful deliveries out of 16 in cases of slight or no RD. Accordingly, of the 19 cases diagnosed as having IRDS only 3 were born without complicating factors at birth.

As shown by Gerschlager (11) there is a high incidence of intrauterine infection in cases of early rupture of the fetal membranes. Our findings support this view since in all but one of the 9 cases where there was a history of early rupture of the membranes extensive inflammatory changes were found at autopsy (26).

Prolonged birth asphyxia and hypoxia have been suggested by Harrison *et al.* (12) to be etiological factors leading to massive intracranial hemorrhages in cases of severe IRDS. In the present series however only one of the 4 cases of massive IVH occurring alone was severely asphyxiated at birth. Of the remaining 9 cases of IVH associated with pulmonary findings 8 suffered from moderate or severe birth asphyxia. On the other hand many of the cases had apneic spells before the onset of IPPR and thus the brain hemorrhages may be also caused by hypoxia associated with apnea.

### *Respiratory symptoms*

Irregular breathing including apneic spells during the first days of life is frequently seen in small otherwise symptomless surviving premature infants. Most of the diseases affecting premature infants are also characterized by respiratory signs of varying extent. Thus owing to these findings the series was divided into three categories according to the severity of the respiratory symptoms during the first 12 to 24 hours after birth.

As shown in Table 4 all 12 infants in the category of severe RD suffered from respiratory failure requiring assisted ventilation, all but one died. As also discussed by Adamson and co-workers (1) these infants may be considered as "non salvageable" and thus could be left out of the respirator treatment. By contrast there were 10 cases treated on IPPR of 21 infants with moderate RD and also 10 cases

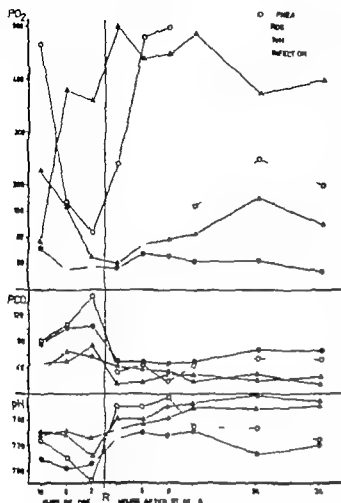


Fig 3 Response to mechanical ventilation changes in arterial  $P_{O_2}$  (determined following a 10 minute period of breathing 90–100% oxygen)  $P_{CO_2}$  and pH following the onset of IPPR in illustrative cases of apnea IRDS IVH and infection (pneumonia)

of 16 with slight or no RD there were 7 and 9 deaths respectively. There is an unexpected high incidence of respiratory failure in infants showing only mild or transient respiratory signs during the first 12 to 24 hours of life. The high mortality rate may be caused by the fact, that in many cases of intrauterine infection (4 cases) and brain hemorrhage (2 cases) the period preceding a sudden attack of respiratory failure requiring IPPR was comparatively symptomless.

Owing to the extreme immaturity of the infants in the present series, the main symptoms of severe IRDS were already indicative of respiratory failure. In contrast to IRDS of the more mature infants there was seldom time enough for the typical respiratory symptom

complex to develop, respirator treatment was started at the mean age of 7 hours. On the other hand there were also cases showing characteristic respiratory symptoms of IRDS but where no pulmonary hyaline membrane could be found at autopsy (26). Of the 6 cases 4 showed only transient signs of IRDS like the cases reported by Downes *et al* (27).

### Blood gases and pH

Initial arterial  $P_{O_2}$  levels in 100% ambient oxygen concentration have been shown by Barton *et al* (4) to be a more reliable guide to prognosis of IRDS than changes in pH and  $P_{CO_2}$ . On the other hand right to left shunt is the dominant cause of hypoxemia (21, 22) and thus the calculated R-L shunt percentage (17) is a useful additional indicator of the pulmonary hypoperfusion (5). Wallgren and co-workers have shown by direct measurement (25) that the extrapulmonary R-L shunt takes place mainly through the foramen ovale rather than ductus arteriosus in premature infants with IRDS.

The infants who died after severe RD in the present series, had a strikingly lower mean  $P_{O_2}$  (7.03)  $P_{O_2}$  (46 mm Hg) and accordingly a high R-L shunt percentage (50) as compared with deaths in the two other categories ( $pH$  7.14 and 7.24  $P_{O_2}$  153 and 297 and R-L shunt \* 27 and 24 respectively). There was only slight difference between  $P_{CO_2}$  values. The comparatively low  $P_{CO_2}$  in the expiring cases of severe IRDS is caused by the fact that many of these infants (5 cases) were already on assisted ventilation when the first arterial samples for the determination of the blood gases and pH were taken.

### Response to IPPR

The response to IPPR as demonstrated by changes in blood gases and pH has been shown to be a valuable prognostic parameter (1–6). This is also true for the extremely small prematures of the present series but only in far as changes of the arterial  $P_{O_2}$  are concerned. As illustrated in Fig 3 there is a rapid rise in  $P_{O_2}$  values of the infants during the first

hemorrhage unrelated to IRDS. These cases are sometimes almost impossible to distinguish from the cases suffering from severe apnea. The cases of intrauterine infection show a moderate response to IPPR initially followed by a later downhill course of the illness. In most cases of severe IRDS no rise in  $P_{50}$  values could be demonstrated.

The only characteristic change in pH and  $P_{50}$  values seems to be the tendency to low  $P_{50}$  and accordingly high pH levels in cases of apnea and IVH. In some cases it is striking enough to be of diagnostic value.

#### Follow up study

A few follow up studies comprising premature infants weighing less than 1250 g at birth have been reported (8-13). Lantieri and co-workers (13) in a retrospective study of low birth weight infants found a high incidence of symptoms indicating brain injury (68%) at the age of 6 to 7 years. However the lack of detailed studies on blood gases and pH, blood glucose levels and even bilirubin make it difficult to draw any conclusions about the possible etiological factors known to be responsible for major neurological damage. On the other hand the intensive care of newborn infants including IPPR might result in increasing the incidence of brain damage by making it possible for the most severely affected infants to survive.

The preliminary data obtained during the follow up study still in progress indicate however that there are only a few infants suffering from severe brain injury. Most of the infants seen at the age of two years are indistinguishable from normal babies. It is still far too early however to draw any conclusions about the survivors because as well known there may be many minor brain injuries which are only possible to find at a later age.

#### SUMMARY

Clinical findings and results of treatment in a series of 49 premature infants with birth weights between 850 and 1250 g are presented.

The "intensive care" included nursing in incubators with high environmental humidity maintaining skin temperature at 36.5°C, umbilical catheterisation (both arterial and venous), intravenous glucose infusions, correction of metabolic acidosis by rapid sodium bicarbonate infusions, oxygen therapy and finally endotracheal intubation and mechanical ventilation.

The series was divided into 3 categories according to the severity of respiratory symptoms. There was only one survivor out of 12 cases in the category of severe RD, whereas 14 of 21 with moderate RD and 9 of 16 with slight or no RD survived. The overall survival rate was 45%. The correlation between clinical findings, arterial blood gases and pH, chest X rays, response to treatment and outcome in each of the 3 categories is discussed. The preliminary data from our follow up studies indicate that there are only a few cases showing signs of permanent brain injury at the age of two years.

#### REFERENCES

1. Adamson T M., Collins L M., Hawker J M. & Reynolds E O R. Mechanical ventilation in newborn infants with respiratory failure. *Lancet* II 227 1964.
2. Avery M E. & Oppenheimer E H. Recent increase in mortality from hyaline membrane disease. *J Pediatr* 57 553 1960.
3. Backstrom L. & Kauppinen M A. The intra-uterine growth as estimated from birth weight data at 25 to 43 weeks of gestation for Finnish babies. *Journal of the Finnish Medical Association* 23 1553 1968.
4. Boston R W., Celler F. & Smith C A. Arterial blood gas tensions and acid base balance in the management of the respiratory distress syndrome. *J Pediatr* 68 74 1964.
5. Chu J., Clements J A., Cotton F., Maus M. H., Sweet A Y., Thomas M A. & Tooley W H. The pulmonary hypoperfusion syndrome. *Pediatrics* 35 733 1965.
6. Cooke R., Indering M., Lombolt N F., Yrjunga M., Zachao-Christiansen B. & Fris-Hansen B. Respiratory failure in the newborn. *Acta Paediatr Scand* 56 498 1967.
7. Downes J I., Arya S., Morrow G. & Boggs T R. Transient respiratory distress syndrome in the newborn. *Arch Dis Child* 42 639 1967.
8. Driffler C M. The incidence of mental and

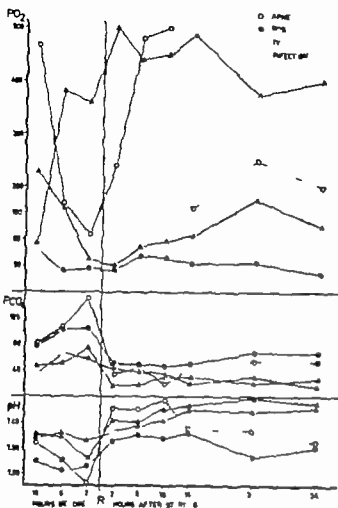


Fig 3 Response to mechanical ventilation changes in arterial  $P_{O_2}$  (determined following a 10 minute period of breathing 90–100% oxygen)  $P_{CO_2}$  and pH following the onset of IPPR in illustrative cases of types IRDS, IVH and infection (pneumonia)

of 16 with slight or no RD there were 7 and 9 deaths respectively. There is an unexpected high incidence of respiratory failure in infants showing only mild or transient respiratory signs during the first 12 to 24 hours of life. The high mortality rate may be caused by the fact, that in many cases of intrauterine infection (4 cases) and brain hemorrhage (2 cases) the period preceding a sudden attack of respiratory failure requiring IPPR was comparatively symptomless.

Owing to the extreme immaturity of the infants in the present series the main symptoms of severe IRDS were already indicative of respiratory failure. In contrast to IRDS of the more mature infants, there was seldom time enough for the typical respiratory symptom

complex to develop, respirator treatment was started at the mean age of 7 hours. On the other hand there were also cases showing the characteristic respiratory symptoms of IRDS but where no pulmonary hyaline membranes could be found at autopsy (26). Of the 6 mild cases 4 showed only transient signs of IRDS like the cases reported by Downes *et al* (7).

### Blood gases and pH

Initial arterial  $P_{O_2}$  levels in 100% ambient oxygen concentration have been shown by Boston *et al* (4) to be a more reliable guide to prognosis of IRDS than changes in pH and  $P_{CO_2}$ . On the other hand right to left shunt is the dominant cause of hypoxemia (21, 22) and thus the calculated R–L shunt percentage (17) is a useful additional indicator of the pulmonary hypoperfusion (5). Wallgren and co-workers have shown by direct measurements (25) that the extrapulmonary R–L shunt takes place mainly through the foramen ovale rather than ductus arteriosus in premature infants with IRDS.

The infants who died after severe RD in the present series had a strikingly lower mean pH (7.03),  $P_{O_2}$  (46 mmHg) and accordingly a higher R–L shunt percentage (50) as compared with deaths in the two other categories (pH 7.14 and 7.24,  $P_{O_2}$  153 and 297 and R–L shunt 27 and 24 respectively). There was only slight if any difference between  $P_{CO_2}$  values. The comparatively low  $P_{CO_2}$  in the expiring cases of severe IRDS is caused by the fact that many of these infants (5 cases) were already on assisted ventilation when the first arterial samples for the determination of the blood gases and pH were taken.

### Response to IPPR

The response to IPPR as demonstrated by changes in blood gases and pH has been shown to be a valuable prognostic parameter (1–6). This is also true for the extremely small prematures of the present series but only as far as changes of the arterial  $P_{O_2}$  are concerned. As illustrated in Fig 3 there is a rapid rise in  $P_{O_2}$  values of the infants dying of brain

## OSTEOBLASTS AND OSTEOCLASTS IN BONE MARROW ASPIRATED FROM CHILDREN WITH RICKETS

YIGAL BARAK

*From the Department of Pediatrics and the Laboratory for Blood Morphology  
and Cytology Kaplan Hospital Rehovoth Israel*

Increased alkaline phosphatase activity in serum (SAP) is the most striking and consistent biochemical abnormality in rickets and forms one of the early changes brought about by Vitamin D deficiency. The rise in SAP tends to be directly related to the severity of the disease and is probably associated with the overproliferation of osteoblasts in the rachitic bones (7).

The present study attempts to demonstrate the osteoblastic proliferation in rickets by simple bone marrow aspiration thus providing an additional diagnostic aid. It also tries to determine whether bone marrow findings are correlated to SAP levels on one hand and to other biochemical and hematological changes characteristic of the disease on the other.

### MATERIAL AND METHODS

A group of 21 children with rickets (Table 1) provided material for the present study. Ten apparently healthy children in the age of 3 to 16 months without rickets formed the control group. Calcium and inorganic phosphorus in serum were determined by the standard methods. Alkaline phosphatase was expressed in Bodansky units.

In 17 cases bone marrow aspiration biopsy was performed from the superior anterior iliac crest using the usual procedure and in the 4 remaining cases from the iliac tubercosity. The aspirated material was examined by phase contrast microscopy and stained with May-Grunwald-Giemsa stains. In 17 cases of rickets smears were stained to demonstrate alkaline phosphatase activity by the Luglow method (8) and in 4 cases staining for acid phosphatase activity was performed according to Kateretzky (11).

Two smears from every case were carefully examined for the counting of osteoblast and osteoclasts but since these cells are small in number and not evenly distributed on the smear counting in relation to other cells seems to provide inaccurate data. Only the total number of osteoblasts and osteoclasts on the whole smear was therefore determined. The cellularity of every smear was estimated by counting the total number of nucleated cells in three measured squares on a graticule in the eyepiece. The mean number of nucleated cells was considered representative of the relative mean smear-cellularity.

### RESULTS

*Osteoblasts* are oval and sometimes elongated cells 25 to 50  $\mu$  in diameter with rather blurred outlines. The cytoplasm in the May-Grunwald-Giemsa stains frequently assumes a light blue shade. It contains few azurophilic granules and is occasionally fenestrated. The nucleus lies eccentrically and a lighter portion (the "perinuclear vacuole") which contains the Golgi bodies (1) is evident in the central part of the cytoplasm. Phase contrast examination revealed that the cytoplasm contains many mitochondria (Fig. 1). *Osteoclasts* are giant cells and their diameter often exceeds 100  $\mu$ . Their outlines are distended and not very clear. The cytoplasm is cloudy and finely granular in the marginal portion and in the May-Grunwald-Giemsa staining it appears from weakly basophilic to strongly acidophilic. It contains numerous azurophilic granules of various sizes and many mitochondria. These cells contain



- physical handicaps in school age children of very low birth weight *Pediatrics* 27 452 1961
- 9 Dunn P M The respiratory distress syndrome of the newborn immaturity versus prematurity *Arch Dis Child* 40 62 1965
  - 10 Fisch R O Gravem H J & Engel R R Neurological status of survivors of neonatal respiratory distress syndrome *J Pediatr* 73 3 1968
  - 11 Gosselin O Contribution à l'étude de l'invasion des organismes maternel et foetal par les microbes des voies genitales inférieure au cours du travail Vaillant Carmanne S A Liege 1945
  - 12 Harrison H Heese de V & Klein M Intracranial hemorrhage associated with hyaline membrane disease *Arch Dis Child* 43 116 1968
  - 13 Kantero R L Ensk M & Holmstrom G Small prematures at 6-7 years of age IV *Ann Paediat Fenn* 11 175 1965
  - 14 Keuth U Das Membransyndrom der Früh- und Neugeborenen Experimentelle Medizin Pathologie und Klinik Band 16 Springer Verlag Berlin
  - 15 Miller H C Respiratory distress syndrome of newborn infants Diagnosis and incidence *J Pediatr* 61 2 1962
  - 16 — Respiratory distress syndrome of newborn infants Clinical study of pathogenesis *J Pediatr* 61 9 1962
  - 17 Nelson N M Prodhom L S Cherry R B Lipsitz P J & Smith C A Pulmonary function in the newborn infant the alveolar arterial oxygen gradient *J Appl Physiol* 18 534 1963
  - 18 Northway W H Rosan R C & Porter D Y Pulmonary disease following respirator therapy of hyaline membrane disease Bronchopulmonary dysplasia *New Eng J Med* 276 7 1967
  - 19 Siggaard Andersen O Engel A Jorgensen A & Astrup P A micro method for determination of pH carbon dioxide tension base excess and standard bicarbonate in capillary blood *Scand J Clin Lab Invest* 12 172 1960
  - 20 Silverman W A & Silverman R H Incidence of hyaline membrane in premature infants *Lancet* II 588 1958
  - 21 Strang L H The pulmonary circulation in the respiratory distress syndrome *Pediat Clin North Amer* 13 3 1966
  - 22 Strang L B & MacLennan M H Ventilatory failure and right to left shunt in newborn infants with respiratory distress *Pediatrics* 28 17 1961
  - 23 Thomas D V Fletcher G Sunshine P Schaffer I A & Klaus M H Prolonged respirator use in pulmonary insufficiency of newborn *JAMA* 193 3 1965
  - 24 Tunstall M E Cater J I Thomson J S & Mitchell R G Ventilating the lungs of newborn infants for prolonged periods *Arch Dis Child* 43 486 1968
  - 25 Wallgren G Hanson J E Tabakin B S Rauha N & Vapaavuori E A Quantitative studies of the human neonatal circulation V Hemodynamic findings in premature infants with and without respiratory distress *Acta Paediatr Scand Suppl* 179 1967
  - 26 Vapaavuori E K & Krohn A Intensive care of small premature infants II Postmortem findings To be published

Submitted June 30 1969

Accepted Oct 29 1969

(E V) Dept of Paediatrics  
University Central Hospital  
Helsinki  
Finland

**Key words** Premature infants intensive care respiratory distress results of treatment



Fig 2 Osteoclast phase contrast microscopy 1,250

ber of osteoblasts in the same smear ( $r = -0.09$  in the minimal values and  $r = +0.18$  in the maximal values). Nor did bone marrow puncture site (tibia or ilium) affect the number of osteoblasts and osteoclasts in the aspirated material.

The ratio between the number of osteoblasts

in bone marrow of the rachitic cases and the level of SAP calcium and phosphorus in their serum was calculated. Significant correlation was found for SAP ( $r = +0.61$ ,  $p < 0.01$ ) (Fig 5). Results for calcium ( $r = -0.28$ ) and phosphorus ( $r = +0.07$ ) however are not significant.

Table 2 Osteoblasts, osteoclasts and relative mean cellularity in bone marrow of the rickets group (I ilium T-tibia)

No	Site of BM aspiration	Nucleated cells per 0.667 mm <sup>2</sup>	Osteoblasts per smear	Osteoclasts per smear
1	I	386-997	30-55	2-3
2	I	478-544	5-7	0-1
3	I	790-874	29-30	2-5
4	I	790-890	17-15	1-2
5	I	277-370	15-20	0-1
6	I	833-836	33-79	10-11
7	T	600-632	19-30	0-2
8	I	166-186	11-11	0-2
9	T	730-880	15-20	0-3
10	T	379-360	42-44	0-2
11	I	140-168	35-45	1-1
12	I	17-190	19-20	1-1
13	T	181-200	15-23	0-2
14	T	210-217	23-32	4-5
15	T	100-112	18-32	1-2
16	I	60-66	51-55	5-8
17	T	460-574	19-26	0-0
18	I	2-8-48	4-7	0-0
19	T	45-50	45-49	0-1
20	T	878-824	30-74	0-2
21	I	124-139	8-10	0-0

Table 1 *Clinical and laboratory Data obtained from 21 Children with Rickets*  
 Ct = cranio-tables Ros = rosary WW = wrist widening)

No	Sex	Age in months	Diagnosis	Clinical signs of Rickets			X Ray wrist	Ca mg/100 ml	P mg/100 ml	Alkal. Phos. (BU)
				Ct	Ros	WW				
M		4.5	Pneumonia	+	+	±	±	10	6.2	27.5
F		8	Anemia Iron def							
			Pneumonia	+	+	+	++	6.0	3.7	20.2
M		10	Anemia Iron def							
			Pneumonia	-	-	+	+	8.2	3.9	22.0
M		7	Cong. cataract	++	-	-	±	11.5	4.4	20.0
F		4.5	Pneumonia	-	+	+	+	10.2	6.3	27.3
			Gastroenteritis							
F		30	Vit D resist rickets	-	++	++	+++	5.7	5.8	83.5
F		4	Pneumonia	+	+	+	±	6.5	4.2	20.0
			Gastroenteritis							
M		7	Mental retardation	-	+	±	±	6.7	4.4	21.7
F		3	Gastroenteritis	+	±	±	+	10.0	3.3	23.2
M		10	Pneumonia Anemia	+	+	±	±	10.0	5.2	37.4
			Iron def							
F		11	Familial	+	-	-	+	6.7	4.1	24.8
			dysautonomia							
M		5.5 yrs	Vit D resist rickets	-	+	+	++	10.2	2.1	26.8
F		7	Pneumonia	+	+	+	+	10.6	3.0	34.6
F		3	Cerebral palsy	+	+	+	±	8.1	5.5	16.5
F		12	Anemia Iron def	++	+	+	++	9.2	2.8	65.5
F		16	Anemia Iron def	±	++	+	+	7.5	2.3	46.6
M		7	Pneumonia	+	±	+	±	8.8	5.7	16.7
F		8	Pneumonia	+	±	±	+	10.0	3.8	14.7
F		8	Pneumonia	+	±	±	+	10.2	4.4	28.7
M		4	Pneumonia Tetany	+	+	+	+	5.4	5.1	40.0
M		4	Pneumonia	-	+	+	+	5.2	6.2	16.0



1 Osteoblast phase contrast microscopy  $\times 1250$

several nuclei which are loosely scattered over the whole cell and do not touch one another (Fig. 2)

Results of osteoblast and osteoclast countings are summarized in Tables 2 and 3. *Osteoblasts* were present in every case of rickets at an average number of 27.19 and ranged from 4 to 79 cells per smear. They were however detected in only 5 cases of the control group and then always less than 4 cells per smear. The increase in number of osteoblasts in rachitic bone marrow is statistically significant (Fig. 3). *Osteoclasts* were present in 19 cases of rickets and ranged from 1 to 11 cells per smear. No osteoclasts were found in the marrow of control subjects.

No correlation could be established between the relative cellularity of smears (expressed by the mean number of nucleated cells per 0.666 mm<sup>2</sup> on 3 regions of the smear) and the num-



Fig. 2. Osteoclast phase contrast microscopy 1,250.

bar of osteoblasts in the same smear ( $r = -0.09$  in the minimal values and  $r = +0.18$  in the maximal values). Nor did bone marrow puncture site (tibia or ilium) affect the number of osteoblasts and osteoclasts in the aspirated material.

The ratio between the number of osteoblasts

in bone marrow of the rachitic cases and the level of SAP calcium and phosphorus in their serum was calculated. Significant correlation was found for SAP ( $r = +0.61$ ,  $p < 0.01$ ) (Fig. 5). Results for calcium ( $r = -0.28$ ) and phosphorus ( $r = +0.07$ ) however are not significant.

Table 2. Osteoblasts, osteoclasts and relative mean cellularity in bone marrow of the rickets group ( $n = 16$  men,  $T = 16$  boys).

No.	Site of BM aspiration	Nucleated cells per 0.666 mm	Osteoblasts per smear	Osteoclasts per smear
1	I	386-597	30-55	2-3
2	I	478-544	5-7	0-1
3	I	790-874	29-30	2-5
4	I	790-850	12-15	1-2
5	I	237-370	15-20	0-1
6	I	833-896	33-79	10-11
7	T	600-611	19-30	0-2
8	I	166-186	11-11	0-2
9	T	730-880	15-20	0-3
10	T	329-360	47-44	0-2
11	I	140-168	35-45	1-1
12	I	172-190	19-70	1-1
13	T	181-700	15-23	0-2
14	T	210-217	23-32	3
15	T	100-112	18-32	1-2
16	I	65-66	34-55	5-8
17	T	460-574	19-26	0-0
18	I	2,8-48	4-7	0-0
19	T	45-50	45-49	0-1
20	T	8-874	30-74	0-2
1	I	1-179	8-10	0-0

Table 3 *Osteoblasts osteoclasts and relative mean cellularity in bone marrow of the control group (I=Ileum T=Tibia)*

No	Site of BM aspiration	Nucleated cells per 0.666 <sup>2</sup> mm <sup>2</sup>	Osteoblasts per smear	Osteoclasts per smear
1	I	200-256	0-0	0
2	T	265-366	0-0	0
3	I	368-441	0-0	0
4	T	720-732	0-2	0
5	T	83-164	0-1	0
6	T	622-872	1-1	0
7	I	52-61	0-1	0
8	I	172-180	1-1	0
9	T	57-80	0-0	0
10	I	400-440	3-4	0

In the 7 cases which were radiologically evaluated as mild rickets the mean number of osteoblasts in the marrow was 24.93 per smear whereas in the 14 cases of florid radiological rickets the average was 28.43 per smear. The *t* test showed that no significant difference exists between these two means.

Osteoblasts consistently showed a positive cytochemical alkaline phosphatase activity in all rachitic marrows. The reaction ranged from few cells with small faintly or moderately stained granules to many cells packed with brilliant brown-black large sized granules (Fig 5). The reaction in osteoclasts was negative. An intense acid phosphatase activity appearing as large red-violet granules with marked diffusion towards the periphery was demonstrated in the osteoclasts (Fig 6). A weak reaction was also found scattered in the osteoblasts.

## DISCUSSION

Osteoblasts and osteoclasts are only rarely found in the bone marrow of healthy infants and adults. Hanick & Libansky (6) aspirated samples of bone marrows from healthy individuals and detected osteoblasts in only 10% and osteoclasts in only 0.7% of the cases. This also confirms the findings of the present study which indicate a highly significant increase in the number of such cells in bone marrow of rachitic children.

Two mechanisms may provide explanation for the above observations. One is related to the fact that the essential lesion of rickets is the accumulation of uncalcified osteoid which is formed by osteoblasts on the surface of the trabeculae in the shaft of the long bones.

In reaction to the muscle stress imposed on the rachitic bones, excessively wide borders of

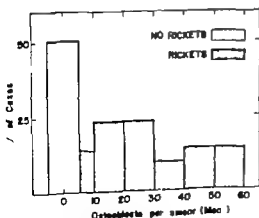


Fig 3 Distribution of osteoblasts per smear of bone marrow in the two groups of children

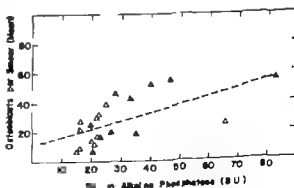


Fig 4 Correlation between SAP level and number of osteoblasts per smear of bone marrow in 21 cases of Rickets

osteoid with plenty of osteoblasts are formed around the trabeculae (7). The abundance of osteoblasts on the inner surface of the bone cortex is probably reflected in the aspirated bone marrow material.

Another mechanism which might contribute to the osteoblastic and osteoclastic proliferation is associated with an increased production of parathyroid hormone stimulated by a reduction in calcium absorption from the intestines (5). There is a rapid proliferation of osteoclasts followed by a compensatory over proliferation of osteoblasts. In extremely severe rachitogenic hyperparathyroidism can be recognized clinically. In such cases (8) characteristic roentgenographic and histologic changes are present in the bone accompanied by massive infiltration of osteoblasts and osteoclasts into the bone marrow.

A literature review revealed only few reports of diseases which are accompanied by high concentration of osteoblasts and osteoclasts in the



Fig. 6. Osteoclast with prominent positive alkaline phosphatase activity. Rosterman method. 1,250.

bone marrow. All the cases reported were characterized by bone destruction and by compensatory bone repair. Rubinstein *et al.* (12) reported 8 cases of osteitis deformans (Paget's disease) in which osteoblasts and osteoclasts were found in abundance in the bone marrow aspirated from affected bones only. This is an obvious finding in such localized condition whereas rickets is a generalized disease. The authors also report the presence of few osteoblasts in the marrow of a patient having bone metastases from a breast cancer as well as in other patients suffering from myeloid leukemia and Thalassemia major.

Hanick & Libensky (6) postulated that numerous osteoblasts and few osteoclasts could be demonstrated in marrow of patients with osteoporosis, metastasizing carcinoma and Paget's disease while few such cells were ob-



Fig. 5. Osteoblast showing a marked positive alkaline phosphatase activity. Kaplan method. 1,250.

Table 3 *Osteoblasts osteoclasts and relative mean cellularity in bone marrow of the control group (I-Ileum T-Tibia)*

No	Site of BM aspiration	Nucleated cells per 0.666 mm <sup>2</sup>	Osteoblasts per smear	Osteoclasts per smear
1	I	200-256	0-0	0
2	T	265-366	0-0	0
3	I	368-441	0-0	0
4	T	720-732	0-2	0
5	T	83-164	0-1	0
6	T	622-872	1-1	0
7	I	52-61	0-1	0
8	I	172-180	1-1	0
9	T	57-80	0-0	0
10	I	400-440	3-4	0

In the 7 cases which were radiologically evaluated as mild rickets the mean number of osteoblasts in the marrow was 24.93 per smear whereas in the 14 cases of florid radiological rickets the average was 28.43 per smear. The *t* test showed that no significant difference exists between these two means.

Osteoblasts consistently showed a positive cytochemical alkaline phosphatase activity in all rachitic marrows. The reaction ranged from few cells with small faintly or moderately stained granules to many cells packed with brilliant brown-black large sized granules (Fig 5). The reaction in osteoclasts was negative. An intense acid phosphatase activity appearing as large red-violet granules with marked diffusion towards the periphery was demonstrated in the osteoclasts (Fig 6). A weak reaction was also found scattered in the osteoblasts.

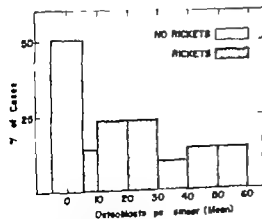


Fig 3 Distribution of osteoblasts per smear of bone marrow in the two groups of children

## DISCUSSION

Osteoblasts and osteoclasts are only rarely found in the bone marrow of healthy infants and adults. Hanick & Libansky (6) aspirated samples of bone marrows from healthy individuals and detected osteoblasts in only 10% and osteoclasts in only 0.7% of the cases. This also confirms the findings of the present study which indicate a highly significant increase in the number of such cells in bone marrow of rachitic children.

Two mechanisms may provide explanation for the above observations. One is related to the fact that the essential lesion of rickets is the accumulation of uncalcified osteoid, which is formed by osteoblasts on the surface of the trabeculae in the shaft of the long bones.

In reaction to the muscle stress imposed on the rachitic bones, excessively wide borders of

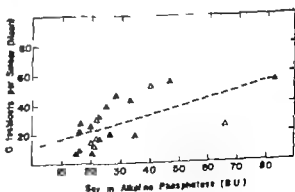


Fig 4 Correlation between SAP level and number of osteoblasts per smear of bone marrow in 21 cases of Rickets

4) osteoid with plenty of osteoblasts are formed around the trabeculae (7). The abundance of osteoblasts on the inner surface of the bone cortex is probably reflected in the aspirated bone marrow material.

Another mechanism which might contribute to the osteoblastic and osteoclastic proliferation is associated with an increased production of parathyroid hormone stimulated by a reduction in calcium absorption from the intestines (5). There is a rapid proliferation of osteoclasts followed by a compensatory over proliferation of osteoblasts. In extremely severe rachitogenic hyperparathyroidism can be recognized clinically. In such cases (8) characteristic roentgenographic and histologic changes are present in the bone accompanied by massive infiltration of osteoblasts and osteoclasts into the bone marrow.

A literature review revealed only few reports of diseases which are accompanied by high concentration of osteoblasts and osteoclasts in the



Fig. 6. Osteoclast with prominent positive acid phosphatase activity. Romanow method 1250.

bone marrow. All the cases reported were characterized by bone destruction and by compensatory bone repair. Rubinstein *et al.* (12) reported 8 cases of osteitis deformans (Paget's disease) in which osteoblasts and osteoclasts were found in abundance in the bone marrow aspirated from affected bones only. This is an obvious finding in such localized condition whereas rickets is a generalized disease. The authors also report the presence of few osteoblasts in the marrow of a patient having bone metastases from a breast cancer as well as in other patients suffering from myeloid leukemia and Thalassemia major.

Hanack & Libansky (6) postulated that numerous osteoblasts and few osteoclasts could be demonstrated in marrow of patients with osteoporosis, metastasizing carcinoma and Paget's disease while few such cells were ob-



Fig. 5. Osteoblast showing a marked positive alkaline phosphatase activity. Kaplan method 1240.



served in case of Hodgkin's disease acute leukemia polycythemia vera and myelofibrosis. They also noted this finding in four cases of rickets without stating the number of cells found. Two other works which were published in European literature (3, 4) report an occasional finding of osteoblasts and osteoclasts in the marrow of few rachitic infants.

Histologic bone sections showed that osteoblasts evince strongly positive alkaline phosphatase activity (10) while osteoclasts show intense acid phosphatase activity (2). This was further confirmed by the present study, due to the increase in numbers of such cells in the rachitic marrow. In contrast to previous observations (2) osteoblasts also showed faint positive acid phosphatase activity.

Results obtained in the course of the present study indicate that the increase in SAP level in rickets is due to osteoblastic proliferation which is reflected in the aspirated bone marrow and in cytochemical staining. This is in accordance with the demonstration accomplished by the starch gel electrophoresis technique showing that a separate isoenzyme of SAP is derived from the osteoblasts and excreted in increased amounts in rickets cases (13). It may therefore be concluded that in cases of unexplained high SAP level an examination of aspirated bone marrow for presence of osteoblasts and osteoclasts which is conducted parallel to the SAP electrophoretic technique may be of value in providing confirmatory evidence for the osteoblastic origin of high SAP thus pointing to the diagnosis of metabolic bone disorder.

### SUMMARY

Examinations of bone marrow samples aspirated from 21 infants with symptoms of rickets showed a significant increase in the number of osteoblasts and osteoclasts as compared with control material.

The osteoblasts showed marked positive alkaline phosphatase staining reaction while the osteoclasts—and osteoblasts to a lesser extent—showed positive staining reaction of acid phosphatase.

A significant correlation could be established

between number of osteoblasts in bone marrow smears and level of serum alkaline phosphatase.

Previous reports of similar marrow findings in diseases characterized by bone destruction and compensatory bone repair are reviewed. The mechanisms and the possible significance of such findings are evaluated.

### REFERENCES

1. Arnott H J & Paulard F G E. Osteoblastic function and fine structure. *Israel J Med Sci* 3: 657 1967.
2. Burstone M S. Histochemical demonstration of acid phosphatase activity in osteoclasts. *J Histochem Cytochem* 7: 39 1959.
3. Cuzal P & Bertrano L. Les cellules de la zone osseuse du cartilage de conjugaison dans le myelogramme tibial de l'enfant. *Rev Hemat* 1: 293 1946.
4. Esner M. Osteoblasten und Osteoclasten im Knochenmarkstrichen. *Helvet Med Acta* 12: 265 1945.
5. Fanconi G. Physiology and pathology of calcium and phosphate metabolism. *Advances Pediatr* 12: 307 1962.
6. Hanick Z & Libinsky J. The cytomorphology of osteoblasts and osteoclasts. *Acta Haemat* 21: 366 1959.
7. Harrison M E. Rickets. In *Brenneman's Practice of Pediatrics* W F Prior Co Inc 1963 vol 1 chap 36.
8. Illig R, Uehlinger E & Prader A. Sekundärer Hyperparathyroidismus bei Vitamin D-Mangel Rachitis. *Helvet Pediatr Acta* 14: 566 1959.
9. Kaplan L S. A biochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow. *Blood* 10: 1023 1955.
10. Prichard J J. A cytological and histochemical study of bone and cartilage formation in the rat. *J Anat* 86: 259 1952.
11. Rozenzajn L, Mirshak G & Efrati P. Acid phosphatase activity in normal human blood and bone marrow cells as demonstrated by the AZO dye method. *Acta Haemat* 30: 310 1963.
12. Rubenstein M A, Smelin A & Freedman A L. Osteoblasts and osteoclasts in bone marrow resorption. *Arch Int Med* 92: 684 1953.
13. Triswell H F & Jeffers D M. Isoenzymes of alkaline phosphatase in hepatobiliary and skeletal diseases. *Amer J Clin Path* 40: 349 1963.

Submitted Sept 9 1969

Accepted Dec 16 1969

(Y B) Dept of Paediatrics  
Kaplan Hospital  
P O Box 1 Rehovoth  
Israel

**Key words:** Osteoblasts osteoclasts bone marrow rickets

## FIBRINOLYSIS IN HUMAN FETUSES

H EKELOUND ULLA HEDNER and B ASTEDT

From the Coagulation Laboratory & Department of Pediatrics and the Department of Obstetrics and Gynecology General Hospital Malmö Sweden

Our knowledge of fibrinolysis in the human fetus is incomplete. A few investigations are however on record. Thus Zallhaug *et al* (19) assessed the fibrinolytic activity by measuring the whole blood clot lysis time in 16 fetuses with crown-heel lengths between 9.4 and 25.5 cm. They recorded short times ranging from 20 to 120 min. Some of the components of the fibrinolytic system have been studied by Ambros *et al* (1) and Götlin & Biasucci (8). As to plasminogen the former were not able to demonstrate any measurable amounts in 8 foetuses in the 4th to 8th month of gestation while the latter who used an immunological technique obtained values corresponding to about 20 per cent of the adult level in 13 foetuses in the 10th to 26th week of gestation. As in the inhibitors of fibrinolysis Ambros *et al* found the antiplasmin values to reach adult level already by the 4th month of gestation. Götlin & Biasucci found the  $\alpha_2$ -macroglobulin to range from 17 to 42 per cent of the adult value and the  $\alpha_1$  antitrypsin from 56 to 92 per cent.

Vahlquist *et al* (18) studied the fibrinogen in 8 foetuses with the gestational ages of 15 to 18 weeks and crown-heel lengths between 16 and 28 cm. They found numerically lower values than in full term newborns (median values 0.18 g/100 ml and 0.39 g/100 ml respectively) but regarded the series too small to warrant statistical analysis.

Judging from this brief survey then a more

detailed investigation of the foetal development of the fibrinolytic system would be of interest and might also serve as a basis for further study of the subject in prematures.

### MATERIAL AND METHODS

The material consisted of 74 foetuses (including one pair of twins) obtained by induced abortion. The mothers of 73 were healthy and their pregnancies had been terminated on socio-medical grounds. One pregnancy was terminated because of suspected intrauterine rubella. Since the foetus appeared normal it was included in the material. Neither were any of the other foetuses malformed. Routine pathological examination of the placentae revealed nothing remarkable.

All the foetuses were delivered by abdominal hysterotomy under general anaesthesia with O<sub>2</sub>+N<sub>2</sub>O and fluorothane. Immediately after extraction of the foetus and the placenta the umbilical cord was divided and under sterile precautions a plastic catheter (French No 3 / 1) was introduced into one of the umbilical arteries. Blood was drawn with a disposable plastic syringe. This technique was successful in all but the smallest foetuses where the samples were obtained by heart puncture.

Since some of the samples were small and since we had to share them with other investigators our 33 foetuses were not always large enough to allow performance of the complete set of determinations.

#### Laboratory procedures

The blood was collected in heparinized glass tubes and in disposable plastic tubes. Citrated plasma and serum were prepared in the way described previously (12, 13). The following determinations were made with the methods described earlier (4) except for the antiplasmin assay.

1. Fibrinolytic activity: fibrin plate method (3)
2. Euplokin® clot lysis time (14)

served in case of Hodgkin's disease acute leukemia polycythemia vera and myelofibrosis. They also noted this finding in four cases of rickets without stating the number of cells found. Two other works which were published in European literature (3, 4) report an occasional finding of osteoblasts and osteoclasts in the marrow of few rachitic infants.

Histologic bone sections showed that osteoblasts evince strongly positive alkaline phosphatase activity (10) while osteoclasts show intense acid phosphatase activity (2). This was further confirmed by the present study due to the increase in numbers of such cells in the rachitic marrow. In contrast to previous observations (2) osteoblasts also showed faint positive acid phosphatase activity.

Results obtained in the course of the present study indicate that the increase in SAP level in rickets is due to osteoblastic proliferation which is reflected in the aspirated bone marrow and in cytochemical staining. This is in accordance with the demonstration accomplished by the starch gel electrophoresis technique showing that a separate isoenzyme of SAP is derived from the osteoblasts and excreted in increased amounts in rickets cases (13). It may therefore be concluded that in cases of unexplained high SAP level, an examination of aspirated bone marrow for presence of osteoblasts and osteoclasts which is conducted parallel to the SAP electrophoretic technique may be of value in providing confirmatory evidence for the osteoblastic origin of high SAP thus pointing to the diagnosis of metabolic bone disorder.

### SUMMARY

Examinations of bone marrow samples aspirated from 21 infants with symptoms of rickets showed a significant increase in the number of osteoblasts and osteoclasts as compared with control material.

The osteoblasts showed marked positive alkaline phosphatase staining reaction while the osteoclasts—and osteoblasts to a lesser extent—showed positive staining reaction of acid phosphatase.

A significant correlation could be established

between number of osteoblasts in bone marrow smears and level of serum alkaline phosphatase.

Previous reports of similar marrow findings in diseases characterized by bone destruction and compensatory bone repair are reviewed. The mechanisms and the possible significance of such findings are evaluated.

### REFERENCES

1. Arnott H J & Pantard F G E. Osteoblastic function and fine structure. *Israel J Med Sci* 3: 657 1967.
2. Burnstone M S. Histochemical demonstration of acid phosphatase activity in osteoclasts. *J Histochem Cytochem* 7: 39 1959.
3. Carl P & Bertrano L. Les cellules de la zone ostéode du cartilage de conjugaison dans le myélocrome tibial de l'enfant. *Reu. Hemat* 1: 293 1946.
4. Esser M. Osteoblasten und Osteoclasten im Knochenmarkausstrich. *Helvet Med Acta* 12: 265 1945.
5. Fanconi G. Physiology and pathology of calcium and phosphate metabolism. *Advances Pediatr* 12: 307 1962.
6. Hranick Z & Libransky J. The cytomorphology of osteoblasts and osteoclasts. *Acta Haemat* 21: 366 1959.
7. Harrison M E. Rickets. In *Brenneman's Practice of Pediatrics* W F Prior Co Inc 1963 vol 1 chapt 36.
8. Illig H, Uehlinger E & Prader A. Selbstdarstellung Hyperparathyroidismus bei Vitamin D Mangel Rachitis. *Helvet Pediatr Acta* 14: 566 1959.
9. Kaplow L S. A biochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow. *Blood* 10: 1023 1955.
10. Pritchard J J. A cytological and histochemical study of bone and cartilage formation in the rat. *J Anat* 86: 259 1952.
11. Rozenbaum L, Marzohal G & Efrati P. Acid phosphatase activity in normal human blood and bone marrow cells as demonstrated by the AZO-dye method. *Acta Haemat* 30: 310 1963.
12. Rubinstein M A, Smelin A & Freedman A L. Osteoblasts and osteoclasts in bone marrow aspiration. *Arch Int Med* 92: 684 1953.
13. Truwell H F & Jeffers D M. Isoenzymes of alkaline phosphatase in hepatobiliary and skeletal diseases. *Amer J Clin Path* 40: 349 1963.

Submitted Sept 9 1969

Accepted Dec 16 1969

(Y B) Dept of Paediatrics  
Kaplan Hospital  
P O Box 1 Rehovoth  
Israel

Key words: Osteoblasts, osteoclasts, bone marrow, rickets.

## FIBRINOLYSIS IN HUMAN FOETUSES

II EKELOUND ULLA HEDNER and B ASTEDT

From the Coagulation Laboratory the Department of Paediatrics and  
the Department of Obstetrics and Gynecology General Hospital Malmö Sweden

Our knowledge of fibrinolysis in the human foetus is incomplete. A few investigations are however on record. Thus Ziliacopoulos *et al* (19) assessed the fibrinolytic activity by measuring the whole blood clot lysis time in 16 foetuses with crown-heel lengths between 9.4 and 25.5 cm. They recorded short times ranging from 20 to 120 min. Some of the components of the fibrinolytic system have been studied by Ambrose *et al* (1) and Grün & Bissucci (8). As to plasminogen the former were not able to demonstrate any measurable amounts in 8 foetuses in the 4th to 8th month of gestation while the latter who used an immunological technique obtained values corresponding to about 20 per cent of the adult level in 13 foetuses in the 10th to 26th week of gestation. As to the inhibitors of fibrinolysis Ambrose *et al* found the antiplasmin values to reach adult level already by the 4th month of gestation. Grün & Bissucci found the  $\alpha_2$  macroglobulin to range from 17 to 42 per cent of the adult value and the  $\alpha_1$  antitrypsin from 56 to 92 per cent.

Vahlgren *et al* (18) studied the fibrinogen in 8 foetuses with the gestational ages of 15 to 33 weeks and crown-heel lengths between 16 and 28 cm. They found "numerically lower values than in full term newborns (median values 0.18 g/100 ml and 0.39 g/100 ml respectively) but regarded the series too small for a statistical analysis.

Judging from this brief survey then a more

detailed investigation of the foetal development of the fibrinolytic system would be of interest and might also serve as a basis for further study of the subject in premature.

### MATERIAL AND METHODS

The material consisted of 74 foetuses (including one pair of twins) obtained by induced abortion. The mothers of 73 were healthy and their pregnancies had been terminated on socio-medical grounds. One pregnancy was terminated because of suspected neural tube defect. Since the foetus appeared normal it was included in the material. Neither were any of the other foetuses malformed. Routine pathological examination of the placentae revealed nothing remarkable.

All the foetuses were delivered by abdominal hysterotomy under general anaesthesia with O<sub>2</sub> + N<sub>2</sub>O and fluorothane immediately after extraction of the foetus and the placenta the umbilical cord was divided and under sterile precautions a plastic catheter (French No. 3) was introduced into one of the umbilical arteries. Blood was drawn with a disposable plastic syringe. This technique was successful in all but the smallest foetuses where the samples were obtained by heart puncture.

Since some of the samples were small and since we had to share them with other investigators our aliquots were not always large enough to allow performance of the complete set of determinations.

### Laboratory procedures

The blood was collected in subheparized glass tubes and in disposable plastic tubes. Carotid plasma and serum were prepared in the way described previously (12, 15). The following determinations were made with the methods described earlier (4) except for the antiplasmin assay.

1. Fibrinolytic activity: Fibrin plate method (3)
2. Enoglobulin clot lysis time (14)

served in case of Hodgkin's disease acute leukemia, polycythemia vera and myelofibrosis. They also noted this finding in four cases of rickets without stating the number of cells found. Two other works which were published in European literature (3, 4) report an occasional finding of osteoblasts and osteoclasts in the marrow of few rachitic infants.

Histologic bone sections showed that osteoblasts evince strongly positive alkaline phosphatase activity (10) while osteoclasts show intense acid phosphatase activity (2). This was further confirmed by the present study due to the increase in numbers of such cells in the rachitic marrow. In contrast to previous observations (2) osteoblasts also showed faint positive acid phosphatase activity.

Results obtained in the course of the present study indicate that the increase in SAP level in rickets is due to osteoblastic proliferation which is reflected in the aspirated bone marrow and in cytochemical staining. This is in accordance with the demonstration accomplished by the starch gel electrophoresis technique showing that a separate isoenzyme of SAP is derived from the osteoblasts and excreted in increased amounts in rickets cases (13). It may therefore be concluded that in cases of unexplained high SAP level an examination of aspirated bone marrow for presence of osteoblasts and osteoclasts which is conducted parallel to the SAP electrophoretic technique may be of value in providing confirmatory evidence for the osteoblastic origin of high SAP thus pointing to the diagnosis of metabolic bone disorder.

### SUMMARY

Examinations of bone marrow samples aspirated from 21 infants with symptoms of rickets showed a significant increase in the number of osteoblasts and osteoclasts as compared with control material.

The osteoblasts showed marked positive alkaline phosphatase staining reaction while the osteoclasts—and osteoblasts to a lesser extent—showed positive staining reaction of acid phosphatase.

A significant correlation could be established

between number of osteoblasts in bone marrow smears and level of serum alkaline phosphatase.

Previous reports of similar marrow findings in diseases characterized by bone destruction and compensatory bone repair are reviewed. The mechanisms and the possible significance of such findings are evaluated.

### REFERENCES

1. Arnott H J & Paulard F G E. Osteoblast function and fine structure. *Israel J Med Sci* 3: 657 1967.
2. Burstone M S. Histochemical demonstration of acid phosphatase activity in osteoclasts. *J Histochem Cytochem* 7: 39 1959.
3. Cazal P & Bertrino L. Les cellules de la zone ostéode du cartilage de conjugaison dans le myelogramme tibial de l'enfant. *Rev Hemat* 1: 293 1946.
4. Ester M. Osteoblasten und Osteoclasten im Knochenmarkausstrichen. *Helvet Med Acta* 12: 265 1945.
5. Fancourt G. Physiology and pathology of calcium and phosphate metabolism. *Advances Pediatr* 12: 307 1962.
6. Hanick Z & Libansky J. The cytomorphology of osteoblasts and osteoclasts. *Acta Haemat* 21: 366 1959.
7. Harrison M E. Rickets. In: *Brenneman's Practice of Pediatrics* W F Prior Co Inc 1963 vol 1: chap 36.
8. Illig R, Uehlinger E & Prader A. Sekundärer Hyperparathyroidismus bei Vitamin D-Mangel Rachitis. *Helvet Pediatr Acta* 14: 566 1959.
9. Kaplow L S. A biochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow. *Blood* 10: 1023 1955.
10. Pritchard J J. A cytological and histochemical study of bone and cartilage formation in the rat. *J Anat* 86: 259 1952.
11. Rozenszajn L, Marshak G & Efrati P. Acid phosphatase activity in normal human blood and bone marrow cells as demonstrated by the AZO-dye method. *Acta Haemat* 30: 310 1963.
12. Rubinstein M A, Smelin A & Friedman A L. Osteoblasts and osteoclasts in bone marrow aspiration. *Arch Int Med* 92: 684 1953.
13. Tawell H F & Jeffers H M. Isoenzymes of alkaline phosphatase in hepatobiliary and skeletal diseases. *Amer J Clin Path* 40: 349 1963.

Submitted Sept 9 1969

Accepted Dec 16 1969

(Y B) Dept of Paediatrics  
Kaplan Hospital  
P O Box 1 Rehovoth  
Israel

Key words: Osteoblasts, osteoclasts, bone marrow, rickets.

## FIBRINOLYSIS IN HUMAN FOETUSES

H EKLUND, ULLA HEDNER and B ASTEDT

*From the Coagulation Laboratory of the Department of Paediatrics and the Department of Obstetrics and Gynaecology, General Hospital, Malmö, Sweden*

Our knowledge of fibrinolysis in the human foetus is incomplete. A few investigations are however on record. Thus Zilbass *et al* (19) assessed the fibrinolytic activity by measuring the whole blood clot lysis time in 16 foetuses with crown-heel lengths between 9.4 and 25.5 cm. They recorded short times ranging from 20 to 120 min. Some of the components of the fibrinolytic system have been studied by Ambros *et al* (1) and Githin & Busacca (8). As to plasminogen the former were not able to demonstrate any measurable amounts in 8 foetuses in the 4th to 8th month of gestation while the latter who used an immunological technique obtained values corresponding to about 20 per cent of the adult level in 13 foetuses in the 10th to 26th week of gestation. As to the inhibitors of fibrinolysis, Ambros *et al* found the antiplasmin values to reach adult level already by the 4th month of gestation. Githin & Busacca found the  $\alpha_2$ -macroglobulin in range from 17 to 42 per cent of the adult value and the  $\alpha_1$ -antitrypsin from 56 to 97 per cent.

Vahlquist *et al* (18) studied the fibrinogen in 8 foetuses with the gestational ages of 15 to 28 weeks and crown-heel lengths between 16 and 28 cm. They found "numerically lower values than in full term newborns (median values 0.18 g/100 ml and 0.39 g/100 ml respectively) but regarded the series too small to warrant statistical analysis.

Judging from this brief survey then a more

detailed investigation of the foetal development of the fibrinolytic system would be of interest and might also serve as a basis for further study of the subject in premature.

### MATERIAL AND METHODS

The material consisted of 74 foetuses (including one pair of twins) obtained by induced abortion. The mothers of 73 were healthy and their pregnancies had been terminated on socio-medical grounds. One pregnancy was terminated because of suspected intra-uterine foetal death. Since the foetus appeared normal it was included in the material. Neither were any of the other foetuses malformed. Routine pathological examination of the placentae revealed nothing remarkable.

All the foetuses were delivered by abdominal hysterotomy under general anaesthesia with O<sub>2</sub> + N<sub>2</sub>O and fluorothane. Immediately after extraction of the foetus and the placenta the umbilical cord was divided and under sterile precautions a plastic catheter (French No 3) was introduced into one of the umbilical arteries. Blood was drawn with a disposable plastic syringe. This technique was successful in all but the smallest foetuses where the samples were obtained by heart puncture.

Since some of the samples were small and since we had to share them with other investigators our aliquots were not always large enough to allow performance of the complete set of determinations.

#### Laboratory procedures

The blood was collected in siliconized glass tubes and in disposable plastic tubes. Citrated plasma and serum were prepared in the way described previously (12, 15). The following determinations were made with the methods described earlier (4) except for the antiplasmin assay.

1. Fibrinolytic activity: fibrin plate method (3)
2. Englobin clot lysis time (14)

Table 1 *Distribution of the material*

Reported pregnancy week	Crown-heel length	No of foetuses	Plasminogen	Anti plasmin	α <sub>2</sub> macroglobulin	No of determinations of					Fibrinolytic split products serum	serum EACA
						Inhib of urokinase activ of plasminogen	Fibrinol activity Fibrin plates	Englob clot lysis time				
12-13	10	1	1	—	—	—	—	—	—	—	1	—
13-14	11	1	1	—	—	—	—	—	—	—	—	—
15	13	1	—	1	1	—	—	—	—	1	—	—
17 15 15	14	3	3	—	1	—	—	—	—	2	3	—
17 16	15	2	1	—	1	—	—	—	—	1	1	—
16-17 16 15	16	3	3	—	1	—	1	—	1	1	3	—
19 17 17 16 16	17	5	4	—	1	1	—	—	—	2	4	—
19 18 18 17 16 13	18	7	6	1	2	—	1	—	—	3	6	—
19 19 19 18 16	19	5	5	3	3	—	—	—	—	3	5	—
19 20 19 18 18 18 18	20	6	6	5	5	—	1	—	—	6	6	—
19 19 19 19	21	4	2	2	2	—	—	1	—	2	2	—
20-21 19-20 19-20	22	3	2	2	2	2	—	—	—	2	2	—
20 20 20 19 19 18-19	23	6	6	5	5	3	5	2	—	6	6	—
21 20-21 20 20 20 20												
20 19 20 19-20 19	24	10	10	3	7	3	2	1	—	7	8	—
22 22 20 18-19	25	4	3	4	4	2	3	2	—	4	3	—
20-24 20 20 19	26	4	3	1	1	3	1	—	—	2	3	—
23 21	27	2	1	1	1	1	—	—	—	1	1	—
23 20	28	2	2	2	2	1	1	—	—	2	2	—
24	29	1	1	—	1	—	1	1	—	1	1	—
24 20	30	2	1	2	2	2	—	—	—	2	1	—
23-24	31	1	1	1	1	1	—	—	—	1	1	—
18	32	1	1	1	1	1	1	—	—	1	1	—
Total number		74	63	34	44	20	18	8		32	60	

3 *Fibrinolytic split products (FSP) in serum and in serum from blood collected in a tube with an inhibitor of fibrinolysis (EACA)*—serum EACA—immunochemical method (11) Values above 50 mg/100 ml were noted only as > 50 mg/100 ml since further dilution of the serum does not permit reliable quantitation. Since the difference between the amount of FSP in serum and that in serum EACA reflects the plasminogen activator activity we used the difference between pairs of such samples as a measure of the fibrinolytic activity.

4 *Plasminogen immunochemical method* (4, 6)

5 *α<sub>2</sub>-macroglobulin esterolytic method* (5)

6 *Antiplasmin activity (progressive antiplasmin)*

A fibrin plate method requiring only 0.1 ml of serum was used instead of the caseinolytic method (4). A purified plasmin preparation (Grade A freeze dried 15 Spouri CU/mg protein AB Kabi Stockholm) was used in a dilution of 12.5 Spouri CU/ml. 0.4 ml of this solution was added to the serum sample. After incubation for 10 min 10 μl of each sample was applied to heated fibrin plates prepared according to Nilsson & Olow (14). The size of the lysed area after 18-20 hours in 37°C was taken as a measure of the remaining plasmin activity. Pooled sera from 20

apparently healthy persons were used as control material. A standard curve was obtained by plotting the results obtained simultaneously with various concentrations of plasmin (dil 1/1-100 per cent 1/2-50 per cent 1/4-25 per cent) incubated with 0.9 per cent NaCl. The residual amount of plasmin was read against this curve and the result is expressed as a percentage of that in the pooled normal serum. Normal range 60-140 per cent.

7 *Inhibitors of the urokinase activation of plasminogen clot method* (16)

8 *Fibrinogen spectrophotometric method* (13)

According to this method the fibrinogen values are calculated for a haematocrit of 40 per cent. In this material the haematocrit was estimated in only 7 cases. It ranged from 28 to 47 per cent. The median as well as the mean was 37 per cent. We therefore used the same correction factor.

9 *Total serum protein biuret method*

(11) The haematocrit was determined with a micro capillary centrifuge (Cellokint AB Lars Ljungberg & Co Stockholm).

Figs 1-4 give normal values for newborns (4) and adults.

Fibrinogen	Haematocrit	Total serum protein
—	—	—
—	—	—
—	—	—
—	—	—
—	—	—
—	1	—
—	—	1
2	1	3
—	—	2
—	—	—
—	1	—
—	—	—
—	2	3
4	3	1
1	—	4
1	1	1
1	—	1
1	—	—
—	—	—
—	—	—
—	—	—
—	7	17

#### Statistical method

A rank method was used for measuring the correlations of plasminogen, the inhibitors of fibrinolysis, fibrinogen and total serum protein with the crown-heel lengths of the foetuses. This method was chosen in order to avoid random influence of very low or high absolute values.

## RESULTS

Table 1 gives the reported week of pregnancy at abortion ('menstruation age'), crown-heel (CH) lengths of the foetuses and number of analyses in each 'length group'. Since it is known from experience that information about the duration of pregnancy week is unreliable, especially when given by mothers with large foetuses, the results of all analyses were correlated with the CH lengths.

*Fibrinolytic activity on fibrin plates (Fig. 1)*

was measured in 18 cases. The area lysed by plasma on unheated plates ranged between 38 and 486 mm<sup>2</sup> and by the resuspended euglobulin precipitate between 42 and 608 mm<sup>2</sup>. In four subjects the activity of plasma exceeded that of the euglobulin fraction. There was also a small plasmin activity on heated plates (0–44 mm<sup>2</sup> for plasma, 0–48 mm<sup>2</sup> for resuspended euglobulin precipitate).

*The euglobulin clot lysis time (Fig. 1)* was measured in 8 of the 18 cases. It ranged from 6 to 21 minutes.

*Fibrinolytic split products (FSP)* were studied in 68 foetuses. The determinations were made in serum in 52 and in 'serum EACA' in 60 cases. Pairs of samples were obtained from 44 foetuses. The results are given in Figs 1 and 2.

FSP in serum were found in all cases except one. The amounts ranged between 2 and > 50 mg/100 ml.

Serum EACA contained at most traces of FSP in most cases. 21 samples contained between 1 and 5 mg/100 ml, 6 and 12 mg/100 ml were found in two foetuses where the corresponding values in serum were 50 mg/100 ml and 15 mg/100 ml respectively. It can be questioned whether moderate amounts of FSP found in serum EACA reflect the conditions *in vivo*. Sampling difficulties sometimes delayed transfer of the blood from the catheter and syringe to the tube with EACA. During this interval fibrinolytic activity might have produced FSP before being inhibited by EACA.

*Plasminogen (Fig. 3)* determined in 63 cases ranged from 7.5 to 50 per cent with a median of 20 per cent. The values did not vary significantly with CH length ( $r = +0.06$ ,  $p > 0.10$ ).

*Inhibitors of fibrinolysis  $\alpha_2$ -macroglobulin (Fig. 3)* as assayed in 44 cases ranged from 5 to 63 per cent with a median value of 36 per cent. There was a significant increase of the values with the CH length ( $r = +0.35$ ,  $0.01 < p < 0.05$ ). *Antiplasmin (Fig. 3)* was determined in 34 cases. The range was wide, 29–1000 per cent, owing to the occurrence of two low (29 and 33 per cent) and two extremely high values (580 and 1000 per cent), the latter two in



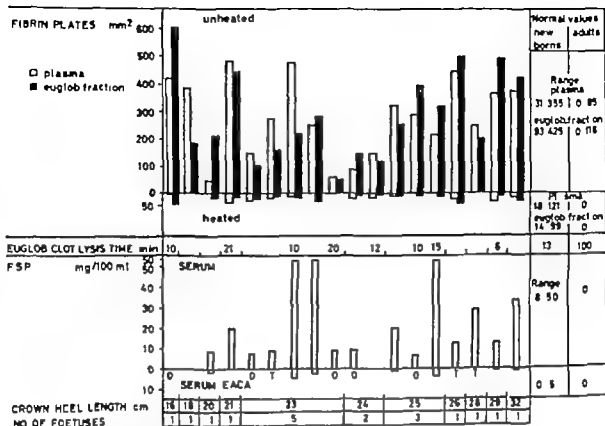


Fig 1 Fibrinolytic activity on fibrin plates in 18 foetuses. Comparison with euglobulin clot lysis time and FSP in some of the cases. 0 = no measurable

amount of activity. T = traces of FSP. -- = determination not done.

the twins. The median was 100 per cent. The antiplasmin did not vary significantly with CH length ( $r = +0.09$ ,  $p > 0.10$ ). Inhibitors of urokinase activation of plasminogen (Fig 3) were estimated in only 20 cases because the method used requires 0.5 ml of serum. The range found

was rather wide (93-768 per cent) with a median of 232 per cent. The values did not vary significantly with CH length ( $r = -0.31$ ,  $p > 0.10$ ).

Fibrinogen (Fig 4) as assayed in 18 cases ranged from 0.03 to 0.31 g/100 ml with a

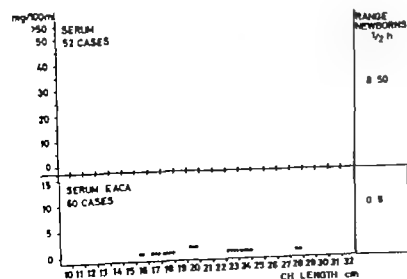


Fig 2 Fibrinolytic split products (mg/100 ml) in serum from 52 foetuses and in serum EACA from 60 foetuses.

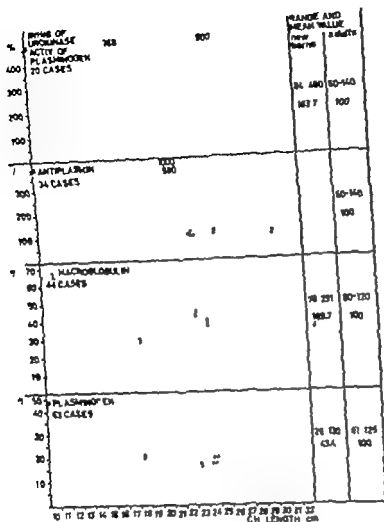


Fig 5 Determinations of plasminogen and the inhibitors of fibrinolysis

median value of 0.09 g/100 ml. The values did not increase with the size of the foetus ( $r = +0.36$   $p > 0.10$ ).

Total serum protein (Fig 5) in 17 cases studied ranged from 3.0 to 4.5 g/100 ml with a median value of 3.4 g/100 ml. It did not vary with CH length ( $r = +0.17$   $p > 0.10$ ).

Haematocrit (Fig 5) was estimated in only 7 cases. The range was 28-47 per cent with a median value of 37 per cent.

## DISCUSSION

We demonstrated the occurrence of plasminogen, plasminogen activator activity, inhibitors

of fibrinolysis and fibrinogen as well as a considerable fibrinolytic capacity in human foetuses from a CH length of 10 cm (corresponding to about 13 weeks of gestation).

We calculated the fibrinolytic activity from lysis on fibrin plates from the euglobulin clot lysis time and from FSP in serum and serum EACA.

As measured on unheated fibrin plates the fibrinolytic activity was considerable. We also found large amounts of FSP in serum but usually in most small amounts in serum EACA. Taken together these findings argue clearly for a high plasminogen activator activity which appeared to be of the same level in all the

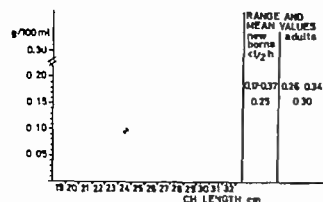


Fig. 4. Fibrinogen values in 18 fetuses.

foetuses studied. The euglobulin clot lysis time was studied in only a few cases. It was short and in agreement with the other findings. The fibrinolytic activity did not increase with the size of the foetus. In most cases the activity reached the same level as that during the first few hours of life in normal newborns (4).

The plasminogen levels measured by an immunochemical method showed a median value of 20 per cent of the adult level without any significant increase during the gestational period studied. Our results agree well with those reported by Gitlin & Biasucci (8) who used radial immunodiffusion for quantitation of the proteins. Ambrus *et al.* (1, 2) were not able to demonstrate any plasminogen at all until shortly before term, apparently because the levels were below the sensitivity of the methods used (2).

The content of inhibitors of plasminogen activation was the same as in newborns (4) and thus larger than in adults.

The level of  $\alpha$  macroglobulin was lower than in newborns (4). It was the only factor in the fibrinolytic system that significantly increased

with the size of the foetus. This variation of  $\alpha$  macroglobulin with postnatal growth is well known (7) but its physiological significance is obscure. Our values agree with those of Gitlin & Biasucci (8).

Progressive antiplasmin was of adult level and did not vary with the size of the foetus. This result agrees with that of Ambrus *et al.* (1) who found antiplasmin at adult levels in the fourth gestational month. Since this antiplasmin activity is located in the  $\alpha_2$  antitrypsin fraction, our results may also be compared with the immunologically quantitated amounts of the protein reported by Gitlin & Biasucci (8). For the corresponding gestational period several values reached 80 to 92 per cent of the adult level. Our results agree fairly well with their determinations.

To conclude, except for  $\alpha$  macroglobulin there was thus an adequate inhibitor level, probably as in the newborns, to counteract the high plasminogen activator activity.

As to fibrinogen, we could not find any data concerning human foetuses since the report of Vahlquist *et al.* (18) in 1953. They studied 8 foetuses (CH lengths 16–28 cm) and found with a spectrophotometric method levels between 0.05–0.29 g/100 ml with a median value of 0.18 g/100 ml. In our 18 cases we found a range between 0.03 and 0.31 g/100 ml with a median value of 0.09 g/100 ml. There was no significant variation with the size of the foetus. Gitlin & Biasucci (8) did not study plasma fibrinogen but demonstrated synthesis of fibrinogen in liver tissue of embryos in the fifth week of gestation.

In conclusion, foetal blood contains plasminogen and a definite plasminogen activator activity which can together produce a remarkable fibrinolytic activity. These findings do not support the hypothesis of Ambrus *et al.* (2) on the pathogenesis of the hyaline membrane syndrome. According to them, the premature infants have virtually no circulating plasminogen and the mechanism for removal of fibrin deposits in alveoli may therefore not function. In our opinion, there is no such deficiency in these in

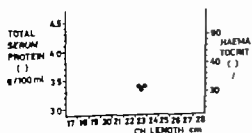


Fig. 5. Total serum protein in 17 foetuses and haematocrit in 7 foetuses.

lants since the fibrinolytic system is already developed. As a matter of fact, Ludwig (10) has produced evidence for enhanced fibrinolysis in hypoxic newborns.

The fibrinolytic mechanism has recently been discussed by Sharp (17). He pointed out the physiologic significance of fibrinolysis in preventing fibrin formation in the circulation.

Since enzyme systems essential to life are active early in the foetus (9, 16) one might tentatively assume that the fibrinolytic system develops very early in order to maintain patency of the foetomaternal circulation and remove fibrin deposits from placental villi. Our investigation has demonstrated a developed fibrinolytic system already in early foetal life possibly to serve this important function.

### SUMMARY

The fibrinolytic system was studied in blood from 74 human foetuses in the 12th to 24th gestational week and with crown heel lengths between 10 and 32 cm.

Plasminogen could be demonstrated in all foetuses in an amount equal to about 20 per cent of that in adults. There was also a definite plasminogen activator activity and a remarkable fibrinolytic capacity. The  $\alpha$ -macroglobulin content was about 30 per cent of that in adults while progressive antiplastin was at and the inhibitors of plasminogen activation above adult level.

The fibrinolytic system develops early in intrauterine life. Like other enzyme systems essential to life it must serve some physiologic purpose, presumably to keep the foetomaternal circulation patent.

### ACKNOWLEDGEMENT

The statistical analysis was carried out by Peter Vorker, F.R.S., Institute of Mathematical Statistics, University of Lund.

This investigation was supported by grants from the Swedish Medical Research Council (870-194, 87 06C) and from the Medical Faculty, University of Lund.

### REFERENCES

1. Ambros C M, Weintrub D H, Niswander J R & Anshus J L. Studies on hyaline membrane disease II. The ontogeny of the fibrinolysin system. *Pediatrics* 35 81 1965.
2. —. Studies on the ontogeny and significance in neonatal disease of the fibrinolysin system and of fibrin stabilizing factor. *Thromb Diath haemorrh* 19 599 1963.
3. Andersson L, Nilsson I M & Olow B. Fibrinolytic activity in man during surgery. *Thromb Diath haemorrh* 7 391 1962.
4. Ekstrand H, Hedner U & Nilsson I M. Fibrinolysis in newborns. *Acta Paediatr Scand* 59 33 1970.
5. Garrot P O. Determination of  $\alpha$ -macroglobulin as trypsin protein esterase. *Chn Chim Acta* 14 493 1966.
6. Garrot P O & Nidein J E. Immunochemical determination of human plasminogen. *Chn Chim Acta* 22 335 1968.
7. Garrot P O & Schersten B. Serum  $\alpha$ -macroglobulin concentration and its variation with age and sex. *Chn Chim Acta* 15 113 1967.
8. Gelin D & Bakketei A. Development of  $\alpha$ -G<sub>1</sub> A yM  $\beta$ - $\beta$  C1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, heparin, fibrinogen, plasminogen, a substrates, orosomucoid  $\beta$  lipoprotein, a macroglobulin and prealbumin in the human conceptus. *J Clin Invest* 48 1435 1969.
9. Kretschmer N, Grossberg R F & Soren F. Biochemical basis of immaturity. *Ann Rev Med* 14 407 1963.
10. Ludwig R. Mikrothrombotische Vorgänge und Thrombozytopenien im fetalen Gehirn bei Hypoxie. *Bull Gynecol* vol 46, Karger, Basel, New York 1968.
11. Nulde J E. Separation and estimation of "split products" of fibrinogen and fibrin in human serum. *Thromb Diath haemorrh* 18 3 1967.
12. Nilsson I M, Blombäck M & von Francken J. On an inherited autosomal hemorrhagic diathesis with antithromphalic globulin (AHG) deficiency and prolonged bleeding time. *Acta Med Scand* 159 35 1957.
13. Nilsson I M & Olow B. Determination of fibrinogen and fibrinogenolytic activity. *Thromb Diath haemorrh* 8 797 1962.
14. —. Fibrinolysis induced by streptokinase in man. *Acta Chir Scand* 123 247 1962.
15. Parakevas M, Nilsson I M & Martinsson G. A method for determining serum inhibitors of plasminogen activation. *Scand J Clin & Lab Invest* 14 138 1966.
16. Soren F & Princely M. The development of enzyme systems. *Ped Clin N Amer* 12 51 1965.
17. Sharp A A. The significance of fibrinolysis. *Proc Roy Soc B* 173 511 1969.
18. Wahlqvist B, Wenberg V & de las Heras J.

Prothrombin and fibrinogen values in the young human fetus *Acta Soc Med Upsal* 58: 281, 1953

- 19 Zilliacus H, Ottelin A. M. & Mattson T. Blood clotting and fibrinolysis in human foetuses *Biol Neonat* 10: 108, 1966

Submitted Dec. 8, 1969

Accepted Dec. 30, 1969

(H. E.) Dept of Paediatrics  
Malmö allmänna sjukhus  
214 01 Malmö  
Sweden

**Key words:** Fibrinolysis, plasminogen inhibitors of fibrinolysis, fibrinolytic split products, fibrinogen, foetus

## DISLOCATION OF THE PROXIMAL EPIPHYSIS OF THE HUMERUS IN NEWBORNS

### Report of Two Cases and Discussion of Diagnostic Criteria

R LEMPERG and B LILJEQUIST

From the Department of Orthopedic Surgery and the Department of Diagnostic Radiology II University of Umeå Umeå Sweden

An early diagnosis of traumatic dislocation of the proximal epiphysis of the humerus in newborns present some difficulties. Traumatic dislocation of the proximal epiphysis of the humerus caused by birth injuries was mentioned by Traesdel (8). One case of extensive caudal dislocation of the epiphysis in relation to the shaft of the humerus was reported by Michel (4). Recently 3 cases were described by Menzies & Rosa (3). Traumatic injury to the epiphysis of the proximal humerus was realized roentgenologically at 15-20 days of age due to a visible callus formation. Scaglietti (5) published 53 cases of birth injuries affecting the proximal epiphysis of the humerus. Apparently only few of these were diagnosed in newborns. In a systematic study of newborns after breech delivery, Snedecor & Wilson (7) noticed epiphyseal dislocations and periosteal stripping at various sites, one of these localized to the proximal part of the humerus. Blount (1) also pointed out the difficulty of early diagnosis and stressed the necessity of repeating the X ray examination after about 1 week in order to detect callus formation.

Two cases of dislocation of the proximal epiphysis of the humerus in newborns are presented.

### CASE REPORTS

**Case 1** Girl born by podalic version and rapid extraction. After birth a paralysis of the left arm of Erb's

type was noticed. An X ray examination of the shoulder joint at this time showed no pathological changes. A certain regress of the paralysis was observed on the seventh day after birth. The same day a new X ray examination was made (Fig. 1). A lateral displacement of the bony epiphyseal nucleus in relation to the humerus shaft was recognized as well as a calcification lateral to the proximal end of the humerus. The X ray examination was interpreted as a luxation of the shoulder joint. An attempt to reduce the luxation under roentgen television control was unsuccessful. Therefore interposition of soft tissue obstructing reposition was considered. On a new examination 3 days later a slight deformity of the contour of the left shoulder area was visible with a slight angulation at the level of the deltoid muscle attachment and a soft tissue swelling around the proximal end of the humerus could be palpated. Spontaneous movements in the shoulder joint was restricted but the biceps muscle showed clear signs of activity. Passive movements in the shoulder joint was normal but obviously evoked pain. Therefore clinically an epiphyseolysis or epiphyseal fracture of the proximal humerus was considered. A new X ray examination 4 days later showed more or less the same findings as in Fig. 1 and was now interpreted as an epiphyseal injury. The arm was placed on an abduction splint for 2 weeks. After this spontaneous movements in the shoulder joint returned and the passive mobility was within normal range. At the same time a new X ray examination revealed abundant callus round the proximal end of the humerus (Fig. 2). No further treatment was given. X ray examination at 6 months of age showed a normal proximal humerus (Fig. 3) with normal clinical findings.

**Case 2** Girl born by podalic version. Immediately after birth a certain angulation was seen of the left upper arm. A soft tissue swelling round the proximal end of the humerus was palpated and a partial paralysis of the Erb type was found. Under careful

Prothrombin and fibrinogen values in the young human fetus *Acta Soc Med Upsal* 58:281, 1953

19. Zilliacus H, Ottelin A-M & Mattson T: Blood clotting and fibrinolysis in human foetuses. *Biol Neonat* 10:108, 1966

Submitted Dec. 8, 1969

Accepted Dec. 30, 1969

(H. E.) Dept of Paediatrics  
Malmö allmänna sjukhus  
214 01 Malmö  
Sweden

**Key words:** Fibrinolysis, plasminogen inhibitors of fibrinolysis, fibrinolytic split products, fibrinogen, foetus.



Fig 4 Case 1 Left (injured) shoulder in inward rotation of the humerus

joints in symmetrical upward and outward rotation (with the elbows flexed to 90°). The importance of the rotational position for demonstration of a dislocation between epiphysis and shaft is shown in Figs 4 and 5. On the right (normal) side the epiphysal nucleus is lying medially to an extension of the shaft of the humerus. On the left (injured) side the lateral dislocation of the epiphysal nucleus in relation to the shaft of the humerus becomes visible.

Dorsal or ventral luxation can best be excluded by axial X-ray whilst a caudal luxation can be seen on frontal pictures. On the other hand an extensively dislocated epiphysal injury without visible nucleus can appear as a caudal luxation when the picture is taken in abduction.

Where no bony nucleus in the epiphysis can

be seen a swelling of the soft parts round the joint and increased distance between the proximal end of the humerus and the scapula can be a sign of bleeding in or around the joint and thereby point to an injury to the epiphysis. Minimum calcium deposits can be visible after some days (Fig 1). According to previous reports even a completely normal X-ray cannot with certainty exclude the presence of an epiphysal injury (1-5).

#### (b) Infants 10-16 days of age

**Clinical symptoms** Swelling and deformity can still be detected whilst the reduction of function or paralysis can show considerable regress. Examination of the shoulder joint can show normal range of movement but can still cause pain.

**X-ray changes** X-ray examination at this stage usually shows callus around the proximal end of the humerus.

The clinical symptoms of an epiphysal injury differ from a pure brachial plexus injury as regards to the visible and palpable deformation of the proximal end of the humerus. The obvious tendency to rapid improvement of active function in the shoulder joint also gives reason to assume that the noticeable decrease in function is more likely to be a restriction due to pain rather than to an organic nerve injury. This interpretation is supported by the fact that



Fig 5 Case 1 Right normal (a) and left, injured (b) shoulder in outward rotation of the humerus with altered relation between epiphysal nucleus and shaft of the humerus on injured side





Fig. 1 Case 1 Left shoulder at 7th day of age. Lateral displacement of bony epiphysis in relation to the shaft of the humerus. Small calcification adjacent to proximal end of humerus (arrow).



Fig. 3 Case 1 Normal left shoulder joint at the age of 1 year.

examination there was a passively free but clearly painful movement of the shoulder joint. An X-ray examination the day after birth showed a slight increase in the distance between the very small but visible epiphyseal nucleus in the proximal humerus and the scapula and a probable injury of the epiphysis was diagnosed. The arm was fixed to the body. A new examination ten days after birth showed a definite regress of the paralysis as well as of the palpable resistance round the proximal end of the humerus. Passive movement in the shoulder joint still seemed to evoke some pain. A new X-ray showed small amounts of callus round the proximal part of the humerus and the bony epiphysis appeared more laterally situated than on the normal side. No further treatment was given. X-ray examination 3 months later showed a lateral dislocation of the bony epiphysis in relation to the shaft of the humerus as well as some deformity and callus remnant round the proximal end of the humerus. The shoulder joint was clinically normal except that a slight angulation of the proximal contour of the upper arm could still be seen.



Fig. 2 Case 1 Left shoulder at 3 weeks of age. Heavy calcified callus around the proximal humerus.

## DISCUSSION

The two above reported cases of traumatic dislocation of the proximal epiphysis of the humerus agree for the most part with the symptomatology described in previous reports. Clinical and X-ray findings which correctly interpreted can help to ensure the diagnosis at an early stage will be summarized and discussed for newborns and for infants above 10 days of age separately.

### (a) Newborns and infants up to 9 days of age

**Clinical symptoms.** Reduced spontaneous movements, pseudo paralysis or paralysis of the arm may be found. These are often of the Erb type. The arm often lies inwardly rotated. Visible deformity and/or palpable swelling round the proximal end of the humerus but with no signs of humerus shaft fracture or noticeable luxation in the humero scapular joint points to an epiphyseal injury. Passive movements in the shoulder joint are obviously painful.

**X-ray changes.** When there is a visible bony nucleus in the epiphysis (present in about 50% of newborns, Garn *et al.* (2)), lateral dislocation in relation to the shaft of the humerus and increased distance to the scapula can be seen. X-ray pictures must be taken of both shoulder

## SERUM HAPTOGLOBIN LEVEL IN DISSEMINATED MALIGNANT DISEASES IN CHILDREN

PETER JOHAN MOE

*From the Department of Paediatrics University of Bergen Bergen Norway*

Haptoglobin is an  $\alpha_2$  glycoprotein which binds hemoglobin. The plasma concentration of this glycoprotein is raised in most diseases associated with destruction of tissue and is decreased in hemolytic disorders. In adults suffering from cancer both high and normal values have been found (4).

The main purpose of this investigation was to assess whether determination of serum haptoglobin could be of differential diagnostic aid in disseminated malignant diseases. In addition it was of interest to see whether different types of drugs influence the haptoglobin level in malignant diseases and whether serum haptoglobin determination could be useful in assessing the activity of the malignant disease.

In addition to quantitative variations haptoglobin also exhibits qualitative variations. It has been suggested that a certain haptoglobin type possibly may be of significance in leukemia (5). Therefore attempts were made in this study to see if certain haptoglobin types were prevalent in disseminated malignant diseases particularly in childhood leukemia.

### MATERIAL AND METHODS

Analyses were performed on serum samples from a total of 140 children: 53 cases of leukemia, 18 cases of other disseminated malignant diseases (8 cases of neuroblastoma, 9 cases of malignant lymphoma and 1 case of Ewing sarcoma), 9 cases of severe infection, 20 cases of benign blood diseases and 35 healthy children. Serum samples from 27 of the leukemia cases were from other hospitals.

Most of the patients from the Children's Hospital have been followed on an outpatient basis after discharge from the hospital. Samples were obtained from most of these cases both prior to and during different types of therapy. All studies have been performed during the last 3 years.

All but one of the patients with malignant diseases and all the 35 healthy children were aged 1 to 13 years.

The drug therapy was divided into the following three types:

- 1 Prednisone therapy
- 2 Combined prednisone and cytostatic therapy
- 3 Cytostatic therapy

The cytostatic drugs used were 6-mercaptopurine, aminopterin, cyclophosphamide, vincristin and Naltrexone.

Serum haptoglobin was measured spectrophotometrically by the method of Tarakowski (6). Duplicate determination on 30 random samples (mean haptoglobin of 137 mg per 100 ml) gave a mean error of the method of 3.7 mg per 100 ml. The error was relatively greater when only small amounts of haptoglobin were present.

Haptoglobin phenotypes were determined by starch electrophoresis by the method of Sævihaug (7).

### RESULTS

Table 1 shows that there is no significant difference in mean haptoglobin level in 40 untreated cases of leukemia and 17 untreated cases of other disseminated malignant disease. Nor was there significant difference between the leukemic cases and 9 children with severe infection.

These determinations were made by Haldis Loe M.D. Institute of Forensic Medicine University of Oslo.

Sever (6) among 1 100 obstetric paralyses of the upper extremity denied the occurrence of epiphyseal injury of the proximal humerus.

The essential factor when estimating the X ray pictures is that the lateral projection of the epiphyseal nucleus is not interpreted as a luxation in the shoulder joint but as a dislocation of the epiphysis.

In analogy with epiphyseal injuries in other long bones this injury can be accompanied by a tearing off of a periosteal bone flap from the metaphysis; periosteal stripping (7). This can be visible on X ray as a small calcium deposit (Fig. 1) and thereby gives an early indication of traumatic epiphyseal injury before a larger callus mass becomes visible.

### CONCLUSION

1 Traumatic injury of the proximal humerus epiphysis especially after delivery in breech position or podalic version should be taken into account in newborn children showing signs of restricted spontaneous function in the shoulder joint or a picture resembling brachial plexus injury.

2 Clinically alteration of the contour of the shoulder area can be seen; movement in the shoulder joint can be painful and swelling round the proximal end of the humerus can be palpated without signs of fracture of the humerus shaft or luxation of the shoulder joint.

3 A diagnosis by means of X ray can be made with certain probability on newborns and up to 9 days of age provided a bony epiphyseal nucleus is present and pictures with outward rotation of the upper arm are taken symmetrically from both sides. Even very small calcium deposits in the soft parts round the proximal end of the humerus can indicate an injury to the epiphysis (periosteal stripping). Negative X ray findings do not exclude epiphyseal injury.

4 X ray examinations should be repeated

between 10–14 days of age on all children with one or several of above mentioned criteria as callus round the proximal end of the humerus can usually be seen in a traumatic epiphyseal injury.

### SUMMARY

Two cases of traumatic dislocation of the proximal humerus epiphysis in newborns are reported. Clinical symptoms for early diagnosis and differentiation from pure brachial plexus injuries are described. Stress is laid on the local findings round the shoulder joint and the importance of taking X ray pictures in suitable symmetrical positions for demonstration of minor dislocations of the epiphyseal nucleus.

### REFERENCES

- 1 Blount W P. *Fractures in children*. Williams & Wilkins, Baltimore, 1955.
- 2 Garn S M, Rohmann C G & Silverman F N. Radiographic standards for postnatal ossification and tooth calcification. *Med Radiogr Photo* 43: 45, 1967.
- 3 Menniti D & Rosen A I. Detachment epifysan. *Obstetrical Arch Path* 21: 192, 1966.
- 4 Michel L. Le décollement ostéocartilagineux de l'épiphyse supérieure de l'humérus. *Rev Chir Orthop* 24: 201, 1937.
- 5 Scaglietti O. The obstetrical shoulder trauma. *Surg Gynec Obstet* 66: 863, 1939.
- 6 Sever J W. Obstetrical paralysis. Report of eleven hundred cases. *JAMA* 85: 1862, 1925.
- 7 Snedecor S T & Wilson H B. Some obstetrical injuries to the long bones. *J Bone Joint Surg* 31A: 378, 1949.
- 8 Truendell E D. *Birth fractures and epiphyseal dislocations*. Paul H Hoeber Inc, New York, 1917.

Submitted Aug. 8, 1969.

Accepted Jan. 22, 1970.

(R. L.) Dept. of Orthopedic Surgery,  
Larsrettet,  
Umeå,  
Sweden.

**Key words:** Epiphyseal separation, proximal humerus, newborns.

## SERUM HAPTOGLOBIN LEVEL IN DISSEMINATED MALIGNANT DISEASES IN CHILDREN

PETER JOHAN MOE

*From the Department of Paediatrics University of Bergen Bergen Norway*

Haptoglobin is an  $\alpha$  glycoprotein which binds haptoglobin. The plasma concentration of this glycoprotein is raised in most diseases associated with destruction of tissue and is decreased in hemolytic disorders. In adults suffering from cancer both high and normal values have been found (4).

The main purpose of this investigation was to assess whether determination of serum haptoglobin could be of differential diagnostic aid in disseminated malignant diseases. In addition it was of interest to see whether different types of drugs influence the haptoglobin level in malignant diseases and whether serum haptoglobin determination could be useful in assessing the activity of the malignant disease.

In addition to quantitative variations haptoglobin also exhibits qualitative variations. It has been suggested that a certain haptoglobin type possibly may be of significance in leukemia (5). Therefore attempts were made in this study to see if certain haptoglobin types were prevalent in disseminated malignant diseases particularly in childhood leukemia.

### MATERIAL AND METHODS

Analyses were performed on serum samples from a total of 140 children: 58 cases of leukemia, 18 cases of other disseminated malignant diseases (8 cases of neuroblastoma, 9 cases of sarcligant lymphoma and 1 case of Ewing sarcoma), 9 cases of severe infection, 20 cases of benign blood diseases and 35 healthy children. Serum samples from 77 of the last 100 cases were from other hospitals.

Most of the patients from the Children's Hospital have been followed on an outpatient basis after discharge from the hospital. Samples were obtained from most of these cases both prior to and during different types of therapy. All studies have been performed during the last 3 years.

All but one of the patients with malignant diseases and all the 35 healthy children were aged 1 to 15 years.

The drug therapy was divided into the following three types:

- 1 Prednisone therapy
- 2 Combined prednisone and cytostatic therapy
- 3 Cytostatic therapy

The cytostatic drugs used were 6-mercaptopurine, aminopterin, cyclophosphamide, vincristin and Nalidixic acid.

Serum haptoglobin was measured spectrophotometrically by the method of Tardif (6). Duplicate determination on 50 random samples (mean haptoglobin of 137 mg per 100 ml) gave a mean error of the method of 3.7 mg per 100 ml. The error was relatively greater when only small amounts of haptoglobin were present.

Haptoglobin phenotypes were determined by starch electrophoresis by the method of Sævihaug (7).

### RESULTS

Table 1 shows that there is no significant difference in mean haptoglobin level in 40 untreated cases of leukemia and 17 untreated cases of other disseminated malignant diseases. Nor was there significant difference between the leukemic cases and 2 children with severe

These determinations were made by Haldis Løv MD Institute of Forensic Medicine University of Oslo

Table 1 Serum haptoglobin concentration in untreated children with leukemia compared with some other diseases

Group		No of cases	Mean haptoglobin mg per 100 ml	s.d.	Range	t Test groups	
I	Leukemia	40	254	111	71-474	I/II	Not significant $p > 0.1$
II	Other malignant dis	17	235	62	115-300		
III	Severe infections	9	279	31	244-309	I/III	Not significant $p > 0.1$
IV	Reticuloendotheliosis	7	127		33-248		
	Mononucleosis	6	30		0-112		
	Osteopetrosis	2	0		0		
	Hemolytic anemia	5	11		0-30		
V	Control group	35	67	20	32-117	I/V & II/V	Significant $p < 0.01$

infections. All three groups had significantly higher mean haptoglobin levels than the controls and the cases of non malignant diseases.

Table 2 shows that high mean haptoglobin level also existed during prednisone therapy.

The mean haptoglobin level in the leukemic cases receiving cytostatic therapy was markedly lower than those observed in untreated cases and in cases receiving prednisone therapy.

Table 3 shows serum haptoglobin values prior to therapy and during prednisone and cytostatic therapy in 12 leukemic cases followed closely by the author for 3 months to 3 years. They represent almost all the cases in Table 2 in whom more than one analysis was performed during therapy. Cases were considered to be in complete remission when they had no clinical or hematological signs of their leukemia. The

table shows that high haptoglobin values also existed during prednisone therapy in cases considered to be in complete remission. Low values were found both while the children received 6 mercaptopurin (mean value of 60 mg per 100 ml) and aminopterin (mean value of 63 mg per 100 ml) therapy. The number of cases is small but there was practically no overlapping between the values during prednisone and cytostatic therapy regardless of presence or absence of remission.

There was a high incidence of haptoglobin values below 30 mg per 100 ml in leukemic cases during cytostatic therapy (Table 3) while none of 35 healthy children had such low values. However no significant difference was found between the two groups (Table 2).

Both high and low values were seen in leu-

Table 2 Serum haptoglobin concentration in children with malignant diseases prior to and during different types of therapy

Group	Therapy	No of cases	No of tests	Mean haptoglobin mg per 100 ml	Range	t Test groups	
I	Leukemia						
	(a) Prior to therapy	40	40	254	71-474	Ia/Ib	Not significant $p > 0.1$
	(b) Prednisone	14	21	226	78-370	Ib/Ic	Significant $p < 0.01$
	(c) Predn + Cytostatic	25	56	143*	0-347		
	(d) Cytostatic	14	40	68	0-145	Ic/Ic	Significant $p < 0.01$
II	Other malign dis						
	(a) Prior to therapy	17	17	235	107-300		
	(b) Prednisone	3	3	242	140-295		
	(c) Cytostatic	12	42	109*	0-300	IIa/IIc	Significant $p < 0.01$
V	Controls	35	35	69	32-117	Ic/V	Not significant $p > 0.1$

\* Mean and t test are calculated from the mean of each case

Table 3 Serum haptoglobin concentration (in mg per 100 ml) in 12 children with leukemia prior to therapy and during prednisone and cytostatic therapy

Patient	Prior to therapy	Prednisone therapy	Cytostatic therapy	
			6-Mercaptopurine	Aminopterin
Bj	136		69 28 18 64	
Fa	189		28	
Gr	315	217 336		
Ha	293	227	60 69 29	
Ho	308	07 225	11 33 17 20 29 33	14 114 88
Iv	245	150	20	20
Jo	572	570 255 371		
Ki	212			70
Li	375	252	126	47 137
Mi	136	117 372 136	134	52 70
Mo	278		79	104
Se	233	78 150	40 10	24 30 20 0 14
Mean	258	230	60	63

Samples during the combined therapy with prednisone and cytostatics are not included in this table

Figures in italics refer to samples during clinical and hematological remission

Mean calculated from the mean of each case

leukemic cases during the combined therapy with prednisone and cytostatics. This group is however too complex for further evaluation.

The mean haptoglobin level during prednisone therapy in the group of other malignant diseases was significantly lower than that observed in untreated cases.

Haptoglobin phenotype studies were performed in a total of 52 cases of malignant diseases including 41 cases of leukemia. The distribution of the three groups corresponded to the distribution in a normal Norwegian population (Table 4).

## DISCUSSION

Serum haptoglobin level seems to be of little differential diagnostic and prognostic value in malignancy in childhood as high values are seen in leukemia, other disseminated malignant diseases and severe infections. Low haptoglobin level seems however to be unlikely in disseminated malignant diseases and is more compatible with a benign type of blood disorder.

Hypohaptoglobinemia during the prednisone therapy may be due to lack of remission. However, high values were also found in leukemia cases in complete remission. It has been shown

that corticosteroids stimulate the production of haptoglobin (2, 3, 6).

The low level in leukemic cases receiving cytostatic therapy may be due to the fact that most of the leukemic cases were in complete remission. However, remission alone can hardly explain the pathological low values observed in this group. It is difficult to evaluate the isolated effect of a cytostatic agent as one cannot give cytostatic therapy to a healthy child and studies on the influence on haptoglobin of cytostatic therapy have not been reported.

It has not been possible to find comparable data in the literature. Turrova (9) and Peacock (5) reported the results of therapy in a

Table 4 Distribution of the haptoglobin groups in disseminated malignant diseases in children

Disease	No. of cases of haptoglobin type			Total no. of cases
	1 1	1 2	2 2	
Leukemia	5	21	15	41
Neuroblastoma	0	3	2	5
Malignant lymphoma	2	2	2	6
Total no. of cases	7	26	19	52
Normal distribution*	132	462	406	1 000

\* Fleischer & Lundevall (11)

few cases of different types of leukemia in adults. But information as to the type of therapy is not given.

Peacock suggested in 1966 (5) that the gene for haptoglobin protein type 2 confers resistance to leukemia on the holders to such an extent that the relative risk of leukemia among homozygous 1-1 as compared with subjects 2-2 is approximately fourfold. Among his 36 cases of lymphoblastic leukemia there were 30.6% haptoglobin type 1-1 and only 16.7% type 2-2. This predominance was not found among our material of 41 studied cases of childhood leukemia: the ratio type 1-1 to 2-2 was 1:3, the same as in a normal Norwegian population. Further studies seem indicated before any conclusions can be drawn as to the possible etiological significance of the serum haptoglobin type.

## SUMMARY AND CONCLUSIONS

Serum haptoglobin studies have been performed in a total of 140 children: 76 cases of disseminated malignant diseases, 9 cases of severe infections, 20 cases of benign blood disorders and 35 healthy controls.

Hyperhaptoglobinemia was found in a large proportion of 40 untreated cases of leukemia and 17 cases of other malignant diseases as well as in 9 cases of severe infections. Serum haptoglobin seems therefore to be of little differential diagnostic value in malignancy in children. Low values are however unlikely in disseminated malignant diseases in children and more compatible with a benign type of blood disorder.

The serum haptoglobin level seems to be dependent not only on the activity of the malignant

disease but also on the type of therapy. The haptoglobin level is therefore of little practical use as a parameter of the activity of a malignant process in childhood.

A normal distribution of serum haptoglobin type was found in 52 cases of disseminated malignant diseases including 41 cases of acute leukemia.

## REFERENCES

1. Fleischer R & Lundevall J. Inheritance of serum groups. *Proc 6th Congr European Soc Haematol* Copenhagen 1957. S. Karger, Basel 1958.
2. Galatus Jensen F. *The haptoglobins. A genetic study*. Dansk Videnskabs Forlag, København 1966.
3. Jayle M F & Bousquet G. Les seromucoides du sang: leur relation avec les mucoprotéines de la substance fondamentale de tissu conjonctif. *Exp. Ann Biochim Med* 17: 757 1955.
4. Nyman M. Serum haptoglobin. Methodological and clinical studies. *Scand J Clin & Lab Invest* Suppl. 39 1959.
5. Peacock A C. Serum haptoglobin type and leukemia: An association with possible etiological significance. *J Nat Cancer Inst* 36: 631 1966.
6. Smith C H. *Blood diseases of infancy and childhood*. C V Mosby Company, St. Louis 1966. 2nd ed.
7. Smithies O. An improved procedure for starch gel electrophoresis in the serum proteins of normal individuals. *Biochem J* 71: 585 1959.
8. Tarukoski P H. Quantitative spectrophotometric determination of haptoglobin. *Scand J Clin & Lab Invest* 18: 90 1966.
9. Turovskiy M G. The effect of therapy on the haptoglobin level in leukemic patients. *Pre Lekarski* 4: 381 1967.

Submitted Nov 1 1969

Accepted Dec 15 1969

Dept of Paediatrics  
Haukeland Sykehus  
5000 Bergen  
Norway

**Key words:** Haptoglobin malignant disease

## BLADDER STONE DISEASE OF CHILDHOOD

### II A Clinico-Pathological Study<sup>1</sup>

A L. AURORA O P. TANEJA and D N GUPTA

From the Departments of Pathology and Surgery *Maxima Aard Medical College and Associated Irwin and G B Pant Hospitals New Delhi India*

Bladder stone disease of childhood continues to be a pediatric problem in South East Asia (1 4 23 35 40 48). Its patchy distribution in Thailand and India suggests the possibility of nutritional factors in its causation. McCarrison (35) on the basis of his experimental work in rats emphasized the importance of Vitamin A and infection in the genesis of bladder stone. However it is well known that bladder stones can form in uninfected urine (34). In recent epidemiological studies in Thailand bladder stones were found in much larger number in the village population. Increased excretion of Calcium and the presence of oxalate crystals in urine was a frequent finding in this population as compared to the urban population of Thailand (24 49). On the other hand in India Andersen *et al* (1) failed to find any significant hypercalcaemia in their patients under the age of 20 years. Thus so far the etiology and pathogenesis of this condition remains obscure. Further the relationship of this condition with the more universally distributed renal calculus has also not been clearly defined.

A clinico-pathological study of 61 cases of bladder stone in childhood was carried out to elucidate the role of factors believed to be responsible in the pathogenesis of this malady.

<sup>1</sup>Partly supported by a grant from the Indian Council of Medical Research.

## MATERIAL AND METHODS

### A Clinical Study

Clinical examination was undertaken with a special emphasis on the detection of any nutritional disorder. Any obstructive uropathy at the time of admission or at operation was recorded.

Hemoglobin estimation, packed cell volume, urea, electrolytes and plain X ray of the entire urinary tract were undertaken. Urine samples for routine and culture studies were obtained directly from the urinary bladder at the time of operation. Intravenous pyelography was undertaken in 16 cases.

### B Biochemical Study

The following biochemical estimations were done in blood and urine:

#### (a) Blood chemistry

1. Serum Calcium (32)
2. Serum uric acid (34)
3. Plasma Vitamins A and carotene using carotene reagent as given by Varley (50)
4. Plasma inorganic phosphorus using stannous chloride (37)
5. Blood urea (32)
6. Plasma proteins total by Biuret method (32) and differential by paper electrophoresis (25)

#### (b) Urine chemistry

1. Estimation of calcium before and after keeping the patient for 4 days on 150 mgms calcium diet, by McCrudden's method as quoted by Varley (50)
2. Oxalate (16) before and after tryptophan load
3. Urochrome (2)
4. Creatinine by Bouncey and Tamsky's method as quoted by Varley (50)
5. Xanthurenic Acid (53) after tryptophan load



few cases of different types of leukemia in adults. But information as to the type of therapy is not given.

Peacock suggested in 1966 (5) that the gene for haptoglobin protein type 2 confers resistance to leukemia on the holders to such an extent that the relative risk of leukemia among homozygous 1-1 as compared with subjects 2-2 is approximately fourfold. Among his 36 cases of lymphoblastic leukemia there were 30.6 haptoglobin type 1-1 and only 16.7% type 2-2. This predominance was not found among our material of 41 studied cases of childhood leukemia, the ratio type 1-1 to 2-2 was 1:3, the same as in a normal Norwegian population. Further studies seem indicated before any conclusions can be drawn as to the possible etiological significance of the serum haptoglobin type.

### SUMMARY AND CONCLUSIONS

Serum haptoglobin studies have been performed in a total of 140 children: 76 cases of disseminated malignant diseases, 9 cases of severe infections, 20 cases of benign blood disorders and 35 healthy controls.

Hyperhaptoglobinemia was found in a large proportion of 40 untreated cases of leukemia and 17 cases of other malignant diseases as well as in 9 cases of severe infections. Serum haptoglobin seems therefore to be of little differential diagnostic aid in malignancy in children. Low values are however unlikely in disseminated malignant diseases in children and more compatible with a benign type of blood disorder.

The serum haptoglobin level seems to be dependent not only on the activity of the malignant

disease but also on the type of therapy. The haptoglobin level is therefore of little practical use as a parameter of the activity of a malignant process in childhood.

A normal distribution of serum haptoglobin type was found in 52 cases of disseminated malignant diseases including 41 cases of acute leukemia.

### REFERENCES

1. Fleischer R & Lundevall J. Inheritance of serum groups. *Proc 6th Congr European Soc Haemat* Copenhagen 1957. S.arger Basel 1958.
2. Galatius Jensen F. *The haptoglobins. A genetic study*. Dansk Videnskabs Forlag, København 1960.
3. Jayle M F & Bourruer G. Les seroconcordances dans leur relations avec les mucoprotéines de la substance fondamentale de tissu conjonctif. *Expos Ann Biochim Med* 17:757 1955.
4. Nymn M. Serum haptoglobin. Methodological and clinical studies. *Scand J Clin & Lab Invest Suppl* 39 1959.
5. Peacock A C. Serum haptoglobin type and leukemia. An association with possible etiological significance. *J Nat Cancer Inst* 36:631 1966.
6. Smith C H. *Blood diseases of infancy and childhood*. C V Mosby Company, St. Louis 1966. 2nd ed.
7. Smithues O. An improved procedure for starch gel electrophoresis in the serum proteins of normal individuals. *Biochem J* 71:585 1959.
8. Tarukoski P H. Quantitative spectrophotometric determination of haptoglobin. *Scand J Clin & Lab Invest* 18:90 1966.
9. Turola M G. The effect of therapy on the haptoglobin level in leukemic patients. *Pre Leukæzi* 4:381 1967.

Submitted Nov 1 1969

Accepted Dec 15 1969

Dept of Pediatrics  
Haukeland Sykehus  
5000 Bergen  
Norway

Key words: Haptoglobin malignant disease

## BLADDER STONE DISEASE OF CHILDHOOD

### II A Clinico Pathological Study<sup>1</sup>

A. L. AURORA, O. P. TANEJA and D. N. GUPTA

*From the Departments of Pathology and Surgery, Maulana A. and Medical College and Associated Institutions and G. B. Pant Hospital, New Delhi, India*

Bladder stone disease of childhood continues to be a pediatric problem in South East Asia (1, 4, 23, 35, 40, 48). Its patchy distribution in Thailand and India suggests the possibility of nutritional factors in its causation. McCarrison (35) on the basis of his experimental work in rats emphasized the importance of Vitamin A and infection in the genesis of bladder stone. However, it is well known that bladder stones can form in uninfected urine (34). In recent epidemiological studies in Thailand, bladder stones were found in much larger number in the village population. Increased excretion of Calcium and the presence of oxalate crystals in urine was a frequent finding in this population as compared to the urban population of Thailand (24, 49). On the other hand, in India, Andersen *et al.* (1) failed to find any significant hypercalcaemia in their patients under the age of 20 years. Thus, so far, the etiology and pathogenesis of this condition remains obscure. Further, the relationship of this condition with the more universally distributed renal calculus has also not been clearly defined.

A clinico-pathological study of 61 cases of bladder stone in childhood was carried out to elucidate the role of factors believed to be responsible in the pathogenesis of this malady.

<sup>1</sup>Partly supported by a grant from the Indian Council of Medical Research.

## MATERIAL AND METHODS

### A. Clinical Study

Clinical estimation was undertaken with a special emphasis on the detection of any nutritional disorder. Any obstructive uropathy at the time of admission or at operation was recorded.

Hemoglobin estimation, packed cell volume, uric acid and plain X-ray of the entire urinary tract were undertaken. Urine samples for routine and culture studies were obtained directly from the urinary bladder at the time of operation. Intravenous pyelography was undertaken in 16 cases.

### B. Biochemical Study

The following biochemical estimations were done in blood and urine:

#### (a) Blood chemistry

1. Serum Calcium (32)
2. Serum creatinine (54)
3. Plasma Vitamin A and carotene using carotene reagent as given by Varley (50)
4. Plasma inorganic phosphorus using stannous chloride (32)
5. Blood urea (32)
6. Plasma protein total by Biuret method (32) and differential by paper electrophoresis (25)

#### (b) Urine chemistry

1. Estimation of calcium before and after keeping the patient for 4 days on 150 mgms calcium diet, by McCruden's method as quoted by Varley (50)
2. Oxalate (16) before and after tryptophan load
3. Uromucoids (21)
4. Creatinine by Rosemeyer and Tarnaky's method as quoted by Varley (50)
5. Xanthurenic Acid (53) after tryptophan load

few cases of different types of leukemia in adults. But information as to the type of therapy is not given.

Peacock suggested in 1966 (5) that the gene for haptoglobin protein type 2 confers resistance to leukemia on the holders to such an extent that the relative risk of leukemia among homozygous 1-1 as compared with subjects 2-2 is approximately fourfold. Among his 36 cases of lymphoblastic leukemia there were 30.6% haptoglobin type 1-1 and only 16.7% type 2-2. This predominance was not found among our material of 41 studied cases of childhood leukemia: the ratio type 1-1 to 2-2 was 1:3, the same as in a normal Norwegian population. Further studies seem indicated before any conclusions can be drawn as to the possible etiological significance of the serum haptoglobin type.

### SUMMARY AND CONCLUSIONS

Serum haptoglobin studies have been performed in a total of 140 children: 76 cases of disseminated malignant diseases, 9 cases of severe infections, 20 cases of benign blood disorders and 35 healthy controls.

Hyperhaptoglobinemia was found in a large proportion of 40 untreated cases of leukemia and 17 cases of other malignant diseases as well as in 9 cases of severe infections. Serum haptoglobin seems therefore to be of little differential diagnostic aid in malignancy in children. Low values are however unlikely in disseminated malignant diseases in children and more compatible with a benign type of blood disorder.

The serum haptoglobin level seems to be dependent not only on the activity of the malignant

disease but also on the type of therapy. The haptoglobin level is therefore of little practical use as a parameter of the activity of a malignant process in childhood.

A normal distribution of serum haptoglobin type was found in 52 cases of disseminated malignant diseases including 41 cases of acute leukemia.

### REFERENCES

1. Flensner R & Lundevall J. Inheritance of serum groups. *Proc 6th Congr European Soc Haematol* Copenhagen 1957. S. Harger, Basel 1958.
2. Galatius Jensen F. *The haptoglobins. A genetic study*. Dansk Videnskabs Forlag, København 1960.
3. Javle M F & Boersier G. Les seromucoides de sang: leur relations avec les mucoprotéines de la substance fondamentale de tissu conjonctif. *Exptl Ann Biochim Med* 17: 757 1955.
4. Nymun M. Serum haptoglobin. Methodological and clinical studies. *Scand J Clin & Lab Invest* Suppl 39 1959.
5. Peacock A C. Serum haptoglobin type and leukemia. An association with possible etiological significance. *J Nat Cancer Inst* 36: 631 1966.
6. Smith C H. *Blood diseases of infancy and childhood*. C V Mosby Company, St. Louis 1966. 2nd ed.
7. Smithies O. An improved procedure for starch gel electrophoresis in the serum proteins of normal individuals. *Biochem J* 71: 585 1959.
8. Tarulinski P H. Quantitative spectrophotometric determination of haptoglobin. *Scand J Clin & Lab Invest* 18: 90 1966.
9. Turovskaja M G. The effect of therapy on the haptoglobin level in leukemic patients. *Pre Lekarski* 4: 381 1967.

Submitted Nov 1 1969

Accepted Dec 15 1969

Dept of Paediatrics  
Haukeland Sykehus  
5000 Bergen  
Norway

**Key words:** Haptoglobin malignant disease

Table 2 Statistical analysis of data on blood chemistry of bladder stone cases

	Calcium (mg per 100 ml)		Vitamin A corrected values (µg per 100 ml)		Bacterococci (log per 100 ml)	
	Patients	Control	Patients	Control	Patients	Control
0-4 years						
Mean	9.57 (22)	8.225 (8)	9.953 (15)	11.625 (4)	17.994 (16)	11.875 (4)
S.D.	0.884	0.436	4.123	3.493	5.367	1.473
Significance	Not		Not		Significant value of $p < 0.05$	
5-9 years						
Mean	9.53 (18)	9.47 (10)	12.05 (16)	18.74 (9)	17.82 (14)	12.758 (5)
S.D.	0.888	0.473	3.834	3.187	7.463	2.565
Significance	Not		Highly significant value of $p < 0.001$		Not	
10-14 years						
Mean	10.116 (6)	9.83 (3)	10.62 (6)	14.80 (3)	15.204 (5)	12.12 (2)
S.D.	0.443	0.711	5.514	4.906	4.416	0.686
Significance	Not		Not		Not	

Values in parentheses indicate the number of cases studied

normal limits and were very much similar in patients and controls

The level of proteins tended to be high in some cases. The values were statistically much higher in the age group 5-9 years with marked increase in beta globulins.

The levels of blood urea ranged from 20 to 86 mg/100 ml with more than 40 mg/100 ml in 9 patients.

#### (c) Urinalysis

The results of chemical, microscopical and bacteriological analysis of urine are summarized in Table 4.

The pH of urine in the majority of the cases was acidic. Protein was present in 26 of the 37 cases investigated. The number of pus cells and red blood cells varied from case to case and did not seem to bear any relation to each

Table 3 Statistical analysis of data on blood chemistry of bladder stone cases

Age group	Phosphorus (mg per 100 ml)		Proteins (g per 100 ml)					
			Total		Albumin		Globulin	
	Patients	Control	Patients	Control	Patients	Control	Patients	Control
0-4 years								
Mean	5.31 (4)	5.938 (4)	8.246 (18)	7.835 (4)	3.836 (17)	3.653 (4)	4.458 (17)	4.18 (4)
S.D.	1.233	0.170	0.899	0.91	0.668	0.237	0.309	0.240
Significance	Not		Not		Not		Not	
5-9 years								
Mean	4.663 (6)	5.199 (9)	9.073 (14)	7.904 (7)	4.303 (14)	3.472 (7)	4.77 (14)	4.34 (7)
S.D.	1.049	0.390	1.049	0.649	0.897	0.609	1.192	0.465
Significance	Not		Highly significant value of $p < 0.02$		Significant value of $p < 0.05$		Not	
10-14 years								
Mean	5.277 (4)	4.977 (3)	10.87 (6)	9.023 (3)	4.678 (6)	4.15 (3)	5.625 (6)	4.871 (1)
S.D.	0.946	0.349	3.617	0.876	1.144	0.078	1.621	0.769
Significance	Not		Not		Not		Not	

Values in parentheses indicate the number of cases studied

*(c) Morphological studies*

This included study of bladder biopsies and bladder stones

## RESULTS

## A Clinical Studies

*(a) History*

80.3% of the cases came from very poor families with an income of less than 150 rupees and the rest had an income ranging from 150 to 400 rupees per month

The staple cereal consumed by the patients was wheat with or without a supplement of jowar, bajra, black gram or rarely rice. Milk, eggs and meat did not form a significant part of the diet. The intake of leafy vegetables was generally low.

In none of these cases a family history of bladder stone was available.

*(b) Symptoms and signs*

The symptoms which brought the patients to the hospital are given in Table 1. The duration of symptoms ranged from 6 days to 8 years. In two cases where the stones were milked out of urethra, the duration of symptoms was only 3 and 4 hours respectively.

The physical signs were few and non-specific. The children showed in general some evidence of growth retardation although no specific signs of deficiency disease were noticeable. Special mention need be made of the absence of ocular signs of Vitamin A deficiency such as Bitot's spots and keratomalacia.

Except for one case with phimosis and an

other with bladder neck obstruction, there was no evidence of obstruction in the lower urinary tract.

## B Laboratory Studies

*(a) Hemoglobin, packed cell volume and absolute values of blood*

All the cases had some degree of iron deficiency anemia as suggested by low mean corpuscular hemoglobin concentration (MCHC). The anemia was slightly more marked in 0-4 years age group as compared to control children. The hemoglobin and MCHC values in the patients were  $10.30 \pm 1.36$  g/100 ml and  $28.82 \pm 1.19$  g/100 ml respectively. The values in control children were  $11.39 \pm 1.19$  g/100 ml and  $30.21 \pm 2.67$  g/100 ml. In 5-9 years and 10-14 years age group there was no difference from the control children of the poor population.

*(b) Blood chemistry*

A statistical analysis of blood constituents is given in Tables 2 and 3. The serum calcium values were similar in the patients and controls.

The plasma Vitamin A (corrected) values ranged between 3.0 to 19.0  $\mu$ g/100 ml in the patients and 8.0 to 29.0  $\mu$ g/100 ml in controls. In the 5-9 years age group the values were significantly lower in patients than in controls.

The value of serum mucoids were higher in patients than in controls and showed a statistical significance in the age group 0-4 years.

The plasma phosphorus levels were within

Table 1 Frequency of symptoms of 61 cases with bladder stone

	Pulling & rubbing of penis in 56 male patients	Pain during micturition	Increased frequency of micturition	Dribbling	Hematuria	Burning micturition	Pain in supra pubic region	Retention of urine
No. of cases	52	44	29	22	19	18	12	2
Percentage of the total	92.67 of the 56 male patients	72.13	47.54	36.06	31.15	29.51	24.59	3.28

Table 5 Statistical analysis of data of urine chemistry in bladder stone cases

Age group	Calcium (mg per 24 hours)		Oxalate (mg per 24 hours)		Xanthonic acid (mg per 24 hours)		Uroscopic acid (mg per 24 hours)	
	Patients	Control	Patients	Control	Patients	Control	Patients	Control
0-4 years								
Mean	63.70 (8)	34.20 (4)	12.47 (9)	12.09 (4)	22.26 (4)	19.93 (4)	99.50 (6)	55.47 (4)
S.D.	43.549	20.10	3.975	3.464	5.03	3.098	21.074	24.88
Significant	Not		Not		Not		Highly significant value of <i>p</i> between 0.02 and 0.01	
5-9 years								
Mean	63.85 (11)	41.31 (6)	17.29 (12)	13.10 (6)	22.54 (6)	18.89 (5)	119.02 (9)	97.74 (6)
S.D.	42.190	19.391	7.470	3.779	9.322	4.159	78.930	32.404
Significant	Not		Not		Not		Not	
10-14 years								
Mean	56.35 (4)	34.29 (2)	20.87 (4)	15.50 (2)	24.27 (3)	18.60 (2)	171.46 (4)	101.54 (2)
S.D.	54.123	10.909	6.580	0.990	5.00	8.025	48.270	3.768
Significant	Not		Not		Not		Not	

Value in parentheses indicate the number of cases studied

0-4 years age group were highly significant when compared with controls. In the other two age groups though the values were higher in patients than in controls but due to wider scatter of the values they were not statistically significant. Thus it would be observed that generally the patients with bladder stone excreted more calcium and more uroscopic acid than the controls children.

#### Radiological examination of patients

Plain X ray of the abdomen including the entire urinary tract was done in each case. In

none of the patients evidence of renal or ureteral stone was present. Intravenous pyelography was carried out in 16 patients. The findings are recorded in Table 6. 31.25% of the patients had no abnormality. 68.75% of the patients had either hydroureter or hydronephrosis or both. The blood urea was higher in patients having bilateral hydroureter and hydronephrosis. It was interesting to note that the degree of proteinuria did not correspond with the radiological changes in the urinary tract.

Table 6 Summary table of intravenous pyelography in 16 cases with bladder stone

	Hydronephrosis		Hydroureter		Hydronephrosis cum hydroureter		No abnormality
	Unilateral	Bilateral	Unilateral	Bilateral	Unilateral	Bilateral	
Number of cases	1	NIL	NIL	2	6	2	5
Percentage of the total number studied (16)	6.25	—	—	12.5	37.5	12.5	31.25
Range of blood urea mg per 100 ml	40	—	—	37 & 44	26 to 40	56 & 86	22 to 40
Mean blood urea mg per 100 ml	—	—	—	40.5	3.66	71	36

Table 4 Summary table of urinalysis of 44 cases with bladder stone

Investigation	Total no of cases studied	No of positive cases in each subgroup of investigation	Culture + VE cases	Culture - VE cases
pH	39			
4 to 4.9		1	1	—
5 to 5.9		18	2	16
6 to 6.9		14	4	10
7 to 8.0		6	3	3
Protein	37			
NIL		11 (NIL)	1	10
Trace to +		20 (9)	5	15
+ ± to ++		6 (4)	3	3
Pus cells	38			
Less than 5 per HPF		24 (6)	1	17
6 to 15 per HPF		9 (5)	1	3
16 to 30 per HPF		2 (1)	NIL	1
More than 30 per HPF		3 (1)	2	NIL
Crystals	38			
Oxalates		1	NIL	1
Phosphates		4	NIL	4
Uric acid & urates		3	NIL	3
Culture	43		13	30

The numbers within parentheses are cases with more than 10 RBC per HPF indicative of hemorrhage  
HPF = high power field

other or to the presence or absence of infection. Of the crystals triple phosphates were present in four cases, uric acid and urates in 3 cases and calcium oxalate in one case. Except in one case where amorphous urates were +++ in none of the cases crystalluria was present to any significant extent.

Out of 43 urine specimens cultured 30 did not reveal any growth. In the remaining 13 specimens *proteus* was isolated in 4, *Escherichia coli* in 3, *Klebsiella* in 2, *Staphylococcus pyogenes* in 2, *Pseudomonas pyocyaneus* in 1 and *Staphylococcus saprophyticus* in 1.

#### (d) Urine chemistry

The values of creatinine were  $0.23 \pm 0.065$  g/24 hours in the patients and  $0.24 \pm 0.033$  g/24 hours in controls and were statistically not significant.

The results of calcium, oxalate, xanthurenic acid and uromucoids are given in Table 5.

The calcium excretion before and after 150 mg of calcium diet failed to show any significant variation. Though the mean values in pa-

tients were 1.1 to 2 times that of similar values in the controls, statistically the difference was not significant due to great variation in the values in the patients. There was no case of hypercalcaemia.

The twenty four hours oxalate excretion in patients before and after tryptophan load failed to show any significant variation between the three age groups and the controls.

The excretion of xanthurenic acid in majority of the patients ranged from 15.92 to 26.74 mg/24 hours. However in two patients in the age group of 5-9 years the values were 29.4 mg and 36.4 mg/24 hours. These patients also tended to excrete a little more oxalate after tryptophan load than before the administration of the amino acid. The possibility of Vitamin B<sub>6</sub> deficiency could not be ruled out in these patients. However statistically the values in all the three age groups were not statistically significantly different from those of the controls.

The 24 hours urinary mucoprotein levels in patients were  $129.99 \pm 49.408$  mg (controls  $33.25 \pm 20.33$  mg). The values in patients of



Fig. 2 Cut surface of type A stone. A central large nodule is surrounded by a thin zone of well or gamed portion formed by laminations.

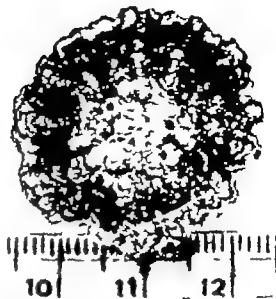


Fig. 4 Cut surface of a type C stone. A large mass of centrally located nuclei is encircled by briefly scalloped laminations.



Fig. 3 Cut surface of a typical type B stone to show the regular pattern of laminations.

der 20 years of age excreted over 200 mg calcium in 24 hours.

About 0.5 to 18.6% of the cases of renal lithiasis are reported to be due to hyperparathyroidism. Niebel & Raorden (39) could find only three cases of bladder stone due to hyperparathyroidism in the world literature including one case reported by them. However, none of these cases was a child. Sanong Unakul has never found a case of hyperparathyroidism related to bladder stone.

In the present series, no case had a 24 hours urine calcium of 200 mg and in 83.3% the values were less than even 100 mg. The calcium metabolism was not evidently disturbed in these patients as again clear from the serum calcium values which were always less than 11.0 mg/100 ml and in no way different from the controls.

Hypophosphatemia has not been seen in the present or the earlier series reported (4, 41).

It is therefore evident that whereas disturbances in calcium metabolism are well recognized and frequently observed in renal lithiasis



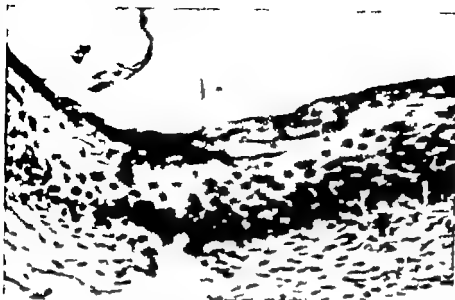


Fig. 1 Histologic section of the urinary bladder stained with hematoxylin and eosin (H & E). The squamoid change in the mucosa with parakeratotic cells on its luminal surface can be made out  $\times 830$ .

### C Morphological studies

#### (a) Bladder biopsies

The changes whenever present were essentially confined to the mucosa and the underlying lamina propria. The lining epithelium of the mucosa was stratified and showed two types of cells, the dark and clear cells (4). The former predominated in 13 biopsies, the latter in 4 and mixed type of epithelium was observed in 13 biopsies. In another two biopsies the ulceration was so extensive that the cell type could not be characterized. It may be mentioned that the dark cells contain large amounts of periodic acid Schiff (PAS) positive material which also forms the greater part of the matrix of stones. There was no relationship between the presence of infection or presence and degree of inflammation and the predominance of any epithelial cell type in the mucosa. However the dark staining cells were seen most frequently with type II stone. Severe grades of ulceration of the epithelium and edema and inflammation of the lamina propria were conspicuous with the spiky type C stones.

In this series in only one biopsy an area of squamoid change without keratinization was seen (Fig. 1). However Vitamin A level in this patient was  $11.5 \mu\text{g}/100 \text{ ml}$ . In several other cases the Vitamin A levels were lower and yet the epithelium did not show such a change.

The stones were classified into three chief types viz A, B and C (Figs 2-4) as reported earlier (4). The type of stone seen in the three age groups is given in the following table.

Stone	Age Group			
	0-4	5-9	10-14	All Ages
A	3	—	—	3
B	28	15	5	48
C	—	8	2	10

Regarding the relationship of type of stone with the presence of infection, urine culture was positive in only 30% of the cases. The positive urine culture was more often associated with type C stones.

The microscopic study of the stones showed features essentially similar to those reported earlier (4, 41).

### DISCUSSION

#### Calcium and phosphorus

Of the urinary mineral abnormalities hypercalciuria has been found to be the most definitive abnormality in renal lithiasis (7, 19, 29, 37, 38). It is however not present in all nor even in majority of the cases. Andersen *et al* (1) reported urinary calcium levels of over 300 mg/day in 87% of their bladder stone cases. However none of their cases un-

Table 7 Differences between renal lithiasis and bladder stone disease of childhood

Parameter	Renal lithiasis	Bladder stone disease of childhood
1. Geographical distribution	World wide	Developing countries viz. South East Asia, India, Pakistan, Southern China, Middle East and North Africa
2. Peak periods of incidence	20 to 50 years	0 to 10 years
3. Incidence by sex Males to females ratio	6:1	33:6:1
4. Socio-economic strata	All strata of society with a slightly greater predominance in the well to-do	Poor population. The condition gradually disappears with general economic and nutritional improvements
5. Recurrence	Not infrequent	Rare
6. History of renal colic	Common	Practically not known
7. Hyperparathyroidism	Reported in about 1% of cases	Not reported
8. Hypercalcaemia	Present in fairly large numbers of cases	Not reported

These renal lithiasis and bladder stone disease of childhood are distinct clinico-pathological disorders with a common gross clinical manifestation of stone formation.

uric acid levels were high in the absence of changes in the kidneys. It need be investigated whether the bladder urothelium of stone cases of childhood contributes some of the Andersen Maclellan material and accounts for high levels of uricacids.

#### Vitamin A deficiency

Enckson & Feldman (18) found defective dark adaptation in 91% of their 61 cases of urolithiasis. However, Aron quoted by Elbot (17) remarked that it takes several months for the Vitamin A body reserves to get exhausted to be reflected in low plasma Vitamin A levels. Despite the absence of Vitamin A deficiency in United States renal lithiasis is fairly common in parts of that country. Conway & co-workers (14) stated that hypovitaminosis A and hypervitaminosis D were rarities in England and yet many cases of urolithiasis were seen there. In Thailand bladder stone disease is fairly common in North East Thailand even though Vitamin A deficiency is seen in all parts of that country (Uthai & Bacobo as quoted by Habibud (23)). In India Vitamin A deficiency is seen in its severest forms in the South where

bladder stone is relatively infrequent as compared to Northern India (13, 42, 43).

Keratinizing metaplasia and subsequent stone formation is seen in rats and guinea pigs on Vitamin A deficient diets (27, 35, 36, 45). This type of metaplastic change in the bladder urothelium in children with bladder stone has not been observed either in the present series or in the earlier one (4, 41).

#### Infection

Helstrom (26), Carrol & Brennan (11), Rose-now (44) and Chute & Soby (12) considered that microorganisms especially ureaproducing bacteria were of great significance in the etiology of urolithiasis. McGeown & Ball (37) on the other hand stated that the presence of urinary infection could not be demonstrated in many cases of renal calculi.

In the present series urine culture was sterile in 70% of the cases. The infection positivity tended to be more frequent with type C stone which had caused severe grades of ulceration of the mucosa and thus had created a good soil for the infection to get implanted. Apparently in these as well as in other cases of

they are practically non existent in primary bladder stone disease of childhood

### Oxalates

Hodgkinson (28) reported hyperoxaluria in a small percentage of cases of renal lithiasis. De Albuquerque & Tuma (15) reported hyperoxaluria in women with recurrent or bilateral renal lithiasis. On the other hand Gershoff & Prien (21) failed to find hyperoxaluria in their cases of recurrent renal lithiasis. Xanthurenic acid excretion on the other hand was significantly greater in their patients than in controls. After tryptophan load 11 of their 18 patients excreted more than 30 mg of xanthurenic acid in 24 hours, an amount which Vilter *et al* (52) consider abnormal. This indicates that Vitamin B<sub>6</sub> deficiency may not always be associated with hyperoxaluria.

Gershoff *et al* (22) and Valyasevi *et al* (49) have reported hyperoxaluria in village boys as compared to city boys in the bladder stone belt of Thailand. This indirectly points to the role of oxaluria in primary bladder stone disease. Significant oxalate crystalluria or increased oxalic acid excretion in 24 hours samples of urine was not seen in the present studies. Only one patient excreted 36.4 mg of xanthurenic acid after tryptophan load.

### Hydrogen ion concentration of urine

It is well known that calcium phosphate and magnesium ammonium phosphate precipitate at a pH of 7.0 or higher whereas calcium oxalate precipitates over the entire physiological pH range. In the present series pH of urine was less than 7.0 in 87.2% of the bladder stone cases. In the entire series crystalluria was negligible or absent. It is obvious that too much emphasis cannot be attached to pH itself in the etiopathogenesis of bladder stone disease of childhood.

### Proteins

The cases of bladder stone disease of childhood show in general some evidence of growth retardation indicated by relatively lower

weights and heights when compared with well fed children. In 15 cases the plasma albumin levels were less than 3.5 g/100 ml. The high globulin levels in these patients bring the total protein values to within normal limits or even higher. The low albumin and high globulin levels were observed in the control children as well. The raised globulin levels in the patients and the controls is probably on account of activity of immune response in childhood.

Aurora *et al* (4) reported raised levels of serum mucoproteins in a small number of cases of bladder stones in children. In the present series the serum mucoproteins in bladder stone cases were higher than control children. This rise is of significance in view of the fact that stone matrix is rich in mucoproteins and thus may be etiologically related.

The important role of urinary mucoproteins in the formation of stone matrix has been highlighted by King & Boyce (33) and Keutel *et al* (31). Boyce *et al* reported 3 to 13 times greater total urinary colloids in all the 17 patients with renal calcigerous stones than in the 14 normal individuals. Keutel (30) remarks that all the fractions of urinary mucoproteins (TNDs) are quite fluctuant in urine from stone formers and that the supranormal values can be found in pathological urine from non stone cases. In the present series, the urinary mucoproteins (the Andersen MacLagan material) levels were raised in the bladder stone cases as compared to controls. Wherever values of seromucoids as well as of uromucoids were available there was no definite correlation. This was to be expected as the site of uromucoids (Tam & Horsfall mucoproteins) formation has been shown to be kidney rather than the blood (31). It need be mentioned that the urinary mucoproteins which contains many substances primarily consist of the Tam & Horsfall mucoprotein (Boyce W H—Personal Communication).

There is no correlation between the values of uromucoids and radiological changes observed after intravenous pyelography in bladder stone cases of present series. At times the



Fig 5 Histological section of a stone to show the multiple round to polyhedral cells having common walls. Each cell has a central area from which threads of matrix extend. H & E 830

aggregate while matrix is an adventitious inclusion a protein adsorbate upon the crystal faces. In the present study the histological examination of some of the stones suggests that the matrix is laid down first as multiple round to polyhedral cells having common walls. In some of the cells the walls become thicker to form the fibrillar matrix (Figs 5 and 6). It is possible that the rate of breakdown of the walls of the cells determines the type of stone. In cases where the walls of the cells persist for long time and increase in thickness the type C stones are formed whereas in instances where the walls break down early with alignment of the remnants of the walls into

fibrillar matrix a type B stone results. When the breakdown is very rapid the matrix is chiefly non fibrillar and a type A stone results. The factors which determine the rate of breakdown of the walls of the cells could be variations in the pH and ionic strength of the urinary constituents besides the physical forces acting during the growth of the stone.

The morphological studies of bladder stone matrices have shown the abundance of PAS positive diastase resistant and Alcian blue negative neutral mucopolysaccharides with a variable mixture of acid mucopolysaccharides (4-41). Aurora & Gupta (3) studying nephrocalcinosis in rabbits have shown that in the

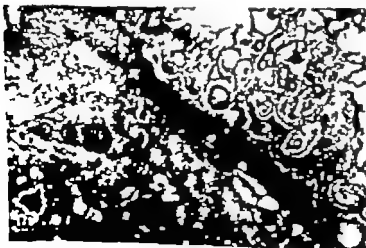


Fig 6 Another area of the stone depicted in Fig 5 to show how the fibrillar and amorphous matrix are laid down (Alcian Blue PAS) 830

stone, infection could be the result rather than the cause

### III Pathogenesis

It has been shown in the preceding paragraphs and highlighted in Table 7 that factors like hypercalcaemia, hyperoxaluria, hyperparathyroidism and infection, which are often held to be responsible for renal lithiasis cannot be incriminated in the problem of bladder stone disease of childhood nor can hypovitaminosis A be implicated. From these observations it is obvious that bladder stone disease of childhood is an entity quite distinct from renal lithiasis.

The questions that now need to be answered are

(a) What is the site of stone formation in bladder stones of childhood? Does the stone descend from the kidneys to grow in the bladder or is it formed in bladder itself?

(b) What is the possible mechanism of formation of the stone?

#### (a) Site of formation of bladder stones

Andersen *et al* (1) have suggested that all bladder stones to begin with are formed in the kidneys. This belief is based on their analysis of 30 stones. It is suggested by them that the nucleus of bladder stone is formed in the kidney and further phosphatic deposits take place in the bladder. If it be so the composition of the nuclei of bladder stones and those of kidney stones should be alike. In their own analysis Andersen *et al* found considerable differences in the composition of bladder stone and kidney stone nuclei. The data of Ranganathan quoted by Andersen *et al* further points to the marked difference in the composition of bladder and renal stones. Moreover it would also be expected at least in some cases of bladder stones that kidneys also show stone formation. The radiological studies of 61 cases of the present series including intravenous pyelography have failed to reveal even a single case where bladder stone disease was associated with kidney stone. The finding of hydronephrosis and/or hydroureter

in 68.7% of cases in which intravenous pyelography was done suggests a back pressure effect on these organs. It was observed that the average duration of symptoms in cases without hydronephrosis was 5.2 months and in those showing hydronephrosis with or without hydroureter it was 11.27 months. The average size of the stone in the two groups was 4.56 and 7.61 cm<sup>3</sup> respectively. Sanong Unakul (personal communication) from Thailand believes that kidney and bladder stones are independent of each other.

The recurrence of renal lithiasis is well documented. Sutherland (46) studied 345 cases of upper urinary tract lithiasis and recorded recurrence in 28% within 2 years, 58.4% within 5 years and 87.6% within 10 years. This is in contrast to what is observed in bladder stone disease of childhood.

Andersen *et al* (1) found only one case of recurrence without any obvious predisposing cause out of 103 patients. Tiwari (47) followed 53 of the 100 cases of bladder stone in children for a period of 4 to 18 months without any evidence of recurrence. Our epidemiological studies (5) also support the absence or rarity of recurrence of bladder stone in children after a surgical removal.

The features mentioned above suggest that the bladder stones in childhood are formed in the bladder rather than in the kidney.

#### (b) Mechanism of formation of bladder stone

The two chief components of stones are the matrix and the minerals. The matrix is present either as fibrillar component or as an amorphous matrix. The minerals are embedded in the amorphous matrix or aligned along the matrix fibrils. The significance of this intimate relationship between the minerals and matrix is a controversial issue. Boyce & co workers (6, 8-10) extensively reported on the stone matrix and have highlighted the importance of the matrix which extracts the salts from the urine to form the stone. On the contrary Fried & Vermeulen (20) and Vermeulen *et al* (51) state that stone formation is basically a crystalline



Fig 5 Histological section of a stone to show the multiple round to polyhedral cellars having common walls. Each cellar has a central area hung from its walls by thin threads of matrix. H & E. 830

aggregate while matrix is an adventitious inclusion a protein adsorbate upon the crystal faces. In the present study the histological examination of some of the stones suggests that the matrix is laid down first as multiple round to polyhedral cellars having common walls. In some of the cellars the walls become thicker to form the fibrillar matrix (Figs 5 and 6). It is possible that the rate of breakdown of the walls of the cellars determines the type of stone. In cases where the walls of the cellars persist for long time and increase in thickness the type C stones are formed whereas in instances where the walls break down early with absorption of the remnants of the walls into

fibrillar matrix a type B stone results. When the breakdown is very rapid the matrix is chiefly non fibrillar and a type A stone results. The factors which determine the rate of breakdown of the walls of the cellars could be variations in the pH and ionic strength of the urinary constituents besides the physical forces acting during the growth of the stone.

The morphological studies of bladder stone matrices have shown the abundance of PAS positive diastase resistant and Alcian blue negative neutral mucopolysaccharides with a variable mixture of acid mucopolysaccharides (4, 41). Aurora & Gupta (3) studying nephrocalcinosis in rabbits have shown that in the

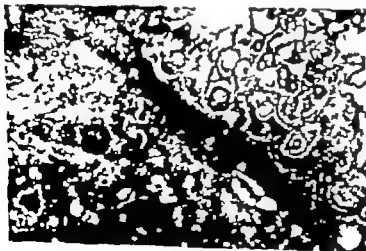


Fig 6 Another area of the stone depicted in Fig 5 to show how the fibrillar and amorphous matrices are laid down (Alcian Blue PAS). 830

stone, infection could be the result rather than the cause

### *III Pathogenesis*

It has been shown in the preceding paragraphs and highlighted in Table 7 that factors like hypercalciuria hyperoxaluria hyperparathyroidism and infection which are often held to be responsible for renal lithiasis, cannot be incriminated in the problem of bladder stone disease of childhood nor can hypovitaminosis A be implicated. From these observations it is obvious that bladder stone disease of childhood is an entity quite distinct from renal lithiasis.

The questions that now need be answered are

(a) What is the site of stone formation in bladder stones of childhood? Does the stone descend from the kidneys to grow in the bladder or is it formed in bladder itself?

(b) What is the possible mechanism of formation of the stone?

#### *(a) Site of formation of bladder stones*

Andersen *et al* (1) have suggested that all bladder stones to begin with are formed in the kidneys. This belief is based on their analysis of 30 stones. It is suggested by them that the nucleus of bladder stone is formed in the kidney and further phosphatic deposits take place in the bladder. If it be so the composition of the nuclei of bladder stones and those of kidney stones should be alike. In their own analysis Andersen *et al* found considerable differences in the composition of bladder stone and kidney stone nuclei. The data of Ranganathan, quoted by Andersen *et al* further points to the marked difference in the composition of bladder and renal stones. Moreover it would also be expected at least in some cases of bladder stones that kidneys also show stone formation. The radiological studies of 61 cases of the present series including intravenous pyelography have failed to reveal even a single case where bladder stone disease was associated with kidney stone. The finding of hydroureter and/or hydronephrosis

in 68.7% of cases in which intravenous pyelography was done suggests a back pressure effect on these organs. It was observed that the average duration of symptoms in cases without hydroureter was 5.2 months and in those showing hydroureter with or without hydronephrosis it was 11.27 months. The average size of the stone in the two groups was 4.56 and 7.61 cm<sup>3</sup> respectively. Sanong Unakul (personal communication) from Thailand believes that kidney and bladder stones are independent of each other.

The recurrence of renal lithiasis is well documented. Sutherland (46) studied 345 cases of upper urinary tract lithiasis and recorded recurrence in 28 within 2 years, 58.4% within 5 years and 87.6% within 10 years. This is in contrast to what is observed in bladder stone disease of childhood.

Andersen *et al* (1) found only one case of recurrence without any obvious predisposing cause out of 103 patients. Tiwari (47) followed 53 of the 100 cases of bladder stone in children for a period of 4 to 18 months without any evidence of recurrence. Our epidemiological studies (5) also support the absence or rarity of recurrence of bladder stone in children after a surgical removal.

The features mentioned above suggest that the bladder stones in childhood are formed in the bladder rather than in the kidney.

#### *(b) Mechanism of formation of bladder stone*

The two chief components of stones are the matrix and the minerals. The matrix is present either as fibrillar component or as an amorphous matrix. The minerals are embedded in the amorphous matrix or aligned along the matrix fibrils. The significance of this intimate relationship between the minerals and matrix is a controversial issue. Boyce & co workers (6-10) extensively reported on the stone matrix and have highlighted the importance of the matrix which extracts the salts from the urine to form the stone. On the contrary Fried & Vermeulen (20) and Vermeulen *et al* (51) state that stone formation is basically a crystalline

- 9 — Crystal Matrix interrelations in calculi *J Urol* 81 351 1959
- 10 Boyce W H, Pool C B, Meehan I & King J S Jr Organic matrix of urinary calculi. Microradiographic comparison of crystalline structure with microscopic and histochemical studies *Acta Radiol* 50 543 1958
- 11 Carroll G & Brennan R V The urea splitting organisms in the formation of urinary calculi *J International College of Surgeons* 17 809 1946
- 12 Chato R & Saby H J Prevalence and importance of urea splitting bacterial infections of the urinary tract in the formation of calculi *J Urol* 44 390 1940
- 13 Coelho O Mortality among children *Indian J Child Health* 4 74 1955
- 14 Conway N S, Marshall A J L & Remon J B The urinary canal excretion in patients with renal calculi *Brit J Urol* 21 30 1949
- 15 De Albuquerque P P & Tente M Investigations on urolithiasis II Studies on oxalate *Trans Amer Ass Gynecol Surg* 53 196 1961
- 16 Dempsey F F, Forbes A P, Mielick R A & Hennessey P H Urinary oxalate excretion *Metabolism* 9 52 1960
- 17 Elliot, J B, Vitman A and Urolithiasis A review *Can J Med* 10 462 1954
- 18 Erickson W J & Feldman J B Signs of Vitamin A deficiency in the eye correlated with urinary ketones. A report of clinical studies and investigations on twenty five patients *JAMA* 169 1706 1957
- 19 Flucka H H Calcium urolithiasis The role of calcium metabolism in the pathogenesis and treatment of calcium urolithiasis *J Urol* 43 214 1949
- 20 Fried F A & Verneulen C W Artificial stone and observations on urea and solubility and supersaturation *Invent Urol* 2 131 1964
- 21 Gerstoft S N & Pines E L Excretion of urinary metabolites in calcium oxalate urolithiasis. Effect of tryptophan and Vitamin B administration *Amer J Clin Nutr* 6 812 1960
- 22 Gerstoft S N, Pines E L & Chandrapanond, A Urinary stones in Thailand *J Urol* 90 285 1963
- 23 Hareend S B Bladder stones in Thailand. A review of the problem *Amer J Trop Med* 10 918 1961
- 24 Hareend S B & Valyaznev A Studies of bladder stone disease in Thailand III Epidemiological studies in Udon Province *Amer J Clin Nutr* 10 1379 1964
- 25 Harrison G A Chemical methods in clinical medicine J & A Churchill Ltd London 1957 pp 43 II
- 26 Hefstrom J Aetiological and therapeutic experiences concerning kidney and ureteric stones *Brit J Urol* 21 9 1949
- 27 Higgins C C Urinary Infection Experimental production and solution with clinical application and end results *J Urol* 36 168 1936
- 28 Hodgkinson A The urinary excretion of oxalate in nephrolithiasis *Proc Roy Soc Med* 51 970 1958
- 29 Hodgkinson A & Pyrah L N The urinary excretion of calcium and inorganic phosphate in 344 patients with calcium stone of renal origin. *Brit J Surg* 46 10 1958
- 30 Keutel H J Fractionation of kidney stone matrix *Urol Int* 19 235 1963
- 31 Keutel H J, King J S Jr & Boyce W H Further studies of urolithiasis in normal and stone urine *Urol Int* 17 3 4 1964
- 32 King H J & Wootton I D P *Microradiography in medical biochemistry* J & A Churchill Ltd London 1959 3rd ed.
- 33 King J S Jr & Boyce W H Immunological studies on serum and urinary proteins in urolithiasis in man *Ann NY Acad Sci* 104 (Art 2) 579 1963
- 34 Lowmley O S & Kewen T J Clinical urology Williams & Williams Co Baltimore 1940 vol II pp 1025-1026
- 35 McCarrison R A lecture on the etiology of stone in India *Brit Med J* 1 1009 1931
- 36 — The experimental production of stones in the bladder Part IV (Indian J Med Res 15 801 1928)
- 37 McKeown M G & Ball G M The pathogenesis of urinary calculus formation *Brit Med J* 15 33 1957
- 38 McIntosh H W & Seragha M Urinary excretion of calcium and citrate in normal individuals and stone formers with variation in calcium intake *Canad Med Ass J* 89 1242 1963
- 39 Nabel J D & Riordan F H Hyperparathyroidism suggested by a primary vesical calculus *Amer J Surg* 97 117 1959
- 40 Pasmore R Observations on the epidemiology of stone in the bladder in Thailand *Lancet* 1 638 1953
- 41 Ramakrishnan V & Ayyar A L Nutritional aspects of calculus disease of the urinary tract *Fed Proc* 20 (No 1 Part III) 317 1961
- 42 Ramakrishnan V Ocular conditions associated with vitamin deficiencies *Antiseptic* 34 591 1957
- 43 Rao P T Customs of infant feeding in South India *Indian Child Health* 6 347 1957
- 44 Rowntree E C Jr Renal calculi. A study of papillary calcification *J Urol* 44 19 1940
- 45 Steiner M, Zuker B & Kramer B Production of renal calculi in guinea pigs by feeding them a diet deficient in Vitamin A *Arch Path* 27 104 1939
- 46 Sutherland J W Recurrence following operations for upper urinary tract stone *Brit J Urol* 26 1954
- 47 Thwait C P Bladder stones in children *Indian Practitioner* 11 1055 1958



early stages there was a gradual thickening of basement membrane of the tubules affected by calcification, due to the deposition of PAS positive material. At a later stage a small amount of Alcian blue positive material was visible in most severely affected tubules. It is possible that a part of the PAS positive material gets converted into Alcian blue positive mucopolysaccharides. In the stone matrices under study a similar change could explain the variable amounts of acid mucopolysaccharides. This is not to suggest that all such material is derived in this fashion and none comes from the urine.

The morphological studies of the bladder biopsies have shown the abundance of PAS positive material in the dark staining cells which predominate in the bladder urothelium of cases of vesical calculi.

It is believed that a wave of change from clear cells to dark cells and back to clear cells is normally present. At times of stress this wave is disturbed so that the dark granular cells do not revert to clear cells till the stress is over. The calcilogenic factors might be acting in a similar fashion increasing the secretion of PAS positive material and thus favouring initiation of stone. What factors or attributes of micro-environment bring about these cellular changes in the urothelium need further study. It appears that neutral mucopolysaccharides elaborated by the dark granular cells under the influence of the calcilogenic factors form a mucus which remains sticking to the mucosal surface till crystalline deposits give it an irregular shape and the stone starts growing.

It is not improbable that there are certain inhibitory substance(s) in the urine of children, the derangement of which by a transiently acting factor brings about the initiation of stone.

### SUMMARY

A clinicopathological study of 61 cases of bladder stone in children was undertaken. The patients showed some evidence of growth fail-

ure. In none of the patients renal or ureteral stone could be visualized.

The serum calcium and plasma phosphorus levels were within normal limits. A significant finding in these cases was raised serum mucoprotein levels. The urinary mucoprotein levels in patients were one and a half to two times higher than in control children. Hypoalbuminemia was observed in 36.6% of the cases.

Morphological studies of bladder biopsies revealed dark granular cells rich in PAS positive material.

From the observations made it has been suggested that the bladder stone disease of childhood is an entity distinct from renal lithiasis. The possible factors in the etiology of the disease have been discussed.

### ACKNOWLEDGEMENTS

Mrs Roche Limited and Mrs Glaxo Limited very kindly supplied Vitamin A standard.

Thanks are due to the Director, Principal, Madras Azad Medical College and Associated Institutions and G. H. Pant Hospitals for permission to publish this work.

### REFERENCES

1. Andersen D. A., Sriramachari S. & Khanna S. K. Investigations into relationship between bladder stones and malnutrition. *Indian J. Med. Sci.* 17: 617, 1963.
2. Anderson A. J. & MacLagan M. F. The isolation and estimation of urinary mucoproteins. *Biochem. J.* 59: 638, 1955.
3. Aurora A. L. & Gupta D. N. Nephrocalcinosis in rabbit. *Indian J. Med. Sci.* 21: 250, 1967.
4. Aurora A. L., Ramalingaswami V. & Gaitonde P. D. Bladder stone disease in children in Delhi area. *J. Urol.* 91: 347, 1964.
5. Aurora A. L., Taneja O. I. & Gupta D. N. Bladder stone disease of childhood. I. An epidemiological study. *Acta Paediatr. Scand.* 59: xxx, 1970.
6. Boyce W. H. & Garvey F. K. The amount and nature of the organic matrix in urinary calculi: a review. *J. Urol.* 76: 213, 1956.
7. Boyce W. H., Garvey F. K. & Goven C. E. Abnormalities of calcium metabolism in patients with idiopathic urinary calculi. Effect of oral administration of sodium phytate. *JAMA* 166: 1577, 1958.
8. Boyce W. H. & King J. E. Jr. Present concepts concerning the origin of matrix and stones. *Ann. NY Acad. Sci.* 104 (Art. 2): 563, 1963.

## SERUM CREATININE VALUES IN HEALTHY CHILDREN

R. A. M. G. DONCKERWOLCKE F. C. SANDER G. J. VAN STEKPLENBURG  
J. W. STOOP and H. A. W. M. TIDDENS

From the Wilhelmina Kinderziekenhuis University Children's Hospital  
Utrecht The Netherlands

Serum creatinine values were determined in a group of 408 children simultaneous with a study of the normal values of serum immunoglobulins (5).

The results appear to be of value in the assessment of serum creatinine levels in patients

containing 1 and 2 mg creatinine per 100 ml. A blank was determined in an identical manner using water instead of the serum specimen.

The results obtained with this method represent the true creatinine values as creatinine free of chromogens is absorbed on Fuller's earth. The scaling down of the sample volume does not affect the results. The precision of the analytical procedure was calculated from (a) duplicate analysis ( $n=34$ ) and (b) the day-by-day variations ( $n=24$ ) of a reference serum (actual concentration 2.5 mg creatinine per 100 ml). Calculation of standard deviation yielded an identical value of  $\pm 5\%$  for both methods.

### MATERIAL AND METHODS

The study involved 408 healthy children (200 boys and 208 girls) aged 4-13 with an equal distribution over the various age groups.

Fasting blood samples were obtained by venipuncture at school. The needles were allowed to clot in glass tubes at room temperature. Within two hours the serum was separated by centrifugation and frozen at  $-20^{\circ}\text{C}$ .

The true creatinine concentration was determined according to De Vries & Daemsleir (6). The volume of the sample was scaled down from 1 ml to 0.05 ml as suggested by Remon van Hage (unpublished) using polyethylene pipettes of the Sear type (4).

The procedure was as follows:

50  $\mu\text{l}$  serum was mixed with 50  $\mu\text{l}$  Fuller's earth suspension (30 mg Fuller's earth in 1 ml 0.36 N HCl) in a microcuvette. After thorough mixing it was allowed to stand for 10 min. 350  $\mu\text{l}$  distilled water was added. After shaking the suspension was centrifuged at 3000 g for 3 min. whereupon the supernatant was decanted. The sediment was washed with 400  $\mu\text{l}$  0.01 N HCl which was discarded after centrifugation.

100  $\mu\text{l}$  0.05 M MgCl<sub>2</sub> solution was added to the sediment and mixed. 100  $\mu\text{l}$  Picrate reagent was added (0.3 g 100 ml picric acid in 0.2 N NaOH) and mixed repeatedly over 70 min. The mixture was then centrifuged at 1000 g for 3 min. The optical density was determined at 514 nm in a microcuvette (optical path length 10 mm volume 120  $\mu\text{l}$ ). Calibration was carried out in duplicate with two standard solutions

### RESULTS

The different values of the serum creatinine concentrations were plotted against age and body surface area. Body surface areas were calculated according to Dubois & Dubois.

We found a wide scattering of the individual creatinine values in relation to age and body surface for both boys and girls (mean values standard deviation range see Table 1). However calculation of the mean values of the creatinine concentrations in relation to age and body surface disclosed a linear relationship (Figs 1 and 2). For the calculation of regression see statistical appendix.

### DISCUSSION

We found a considerable variation in the creatinine values. This cannot be explained by the influence of exercise on serum creatinine. J. B. Henry found that even intensive exercise over

- 48 Unakul S. Urinary stones in Thailand. A statistical survey. *Siriraj Hospital Gazette* 13: 199, 1961.
- 49 Valyasevi A, Halstead S B & Dhanmitta S. Studies of bladder stone disease in Thailand. VI. Urinary studies in children 2-10 years old resident in a Hypo and hyperendemic area. *Amer J Clin Nutr* 20: 1362, 1967.
- 50 Varley H. *Practical clinical biochemistry*. William Heinemann Medical Books Ltd, London, 1958, 2nd ed.
- 51 Vermeulen C W, Lyon E S & Fried F A. On the nature of the stoneforming process. *J Urol* 94: 176, 1965.
- 52 Vilter R W, Mueller J F, Glazer H S, Jr, rold T, Abraham J, Thompson C & Hawkins V R. The effect of Vitamin B deficiency induced by desoxyypyridoxine in human beings. *J Lab Clin Med* 42: 335, 1953.
- 53 Wichstein M & Gudaitis A. Detection of Vitamin B<sub>6</sub> deficiency. Utilization of an improved method for rapid determination of xanthine acid in Urine. *Amer J Clin Path* 72: 652, 1970.
- 54 Weimer H E & Moshin J R. Serum globulin protein concentrations in experimental tuberculosis of guinea pigs. *Amer Rev Tuberculosis* 69: 594, 1953.

Submitted July 16, 1969

Accepted September 9, 1969

(A. L. A.) Department of Pathology

Maulana Azad Medical College

New Delhi 1

India

**Key words:** Bladder stones, children, xanthine, uric acid, hypercalcaemia, hyperoxaluria, bladder biopsy, dark and clear cells, periodic acid Schiff (PAS) positive material, fibrillar matrix, amorphous matrix.

	9	10	11	12
	21	23	33	15
0.485	0.487	0.549	0.560	0.598
0.101	0.101	0.083	0.086	0.112
(0.32-0.64)	(0.31-0.62)	(0.37-0.66)	(0.42-0.78)	(0.41-0.77)
	20	25	31	15
0.479	0.497	0.500	0.562	0.587
0.076	0.083	0.091	0.096	0.084
(0.34-0.60)	(0.34-0.60)	(0.31-0.67)	(0.36-0.84)	(0.49-0.80)
9.200	10.075	10.950	11.825	> 12.700
0.073	10.950	11.823	1.700	
	28	18	15	23
0.504	0.525	0.542	0.572	0.570
0.090	0.105	0.096	0.068	0.103
(0.33-0.69)	(0.35-0.70)	(0.31-0.67)	(0.48-0.73)	(0.39-0.78)
	25	21	18	22
0.506	0.481	0.545	0.507	0.592
0.098	0.113	0.099	0.087	0.078
(0.32-0.69)	(0.31-0.84)	(0.43-0.64)	(0.35-0.67)	(0.47-0.80)

(see statistical appendix). However we might have found differences between the sexes if we had been able to compare the values on the basis of lean body mass.

We publish these results because we have the impression that the graphs in Figs 1 and 2 may have practical significance in the follow up and assessment of creatinine values in individual patients. Longitudinal studies will show whether this impression is correct.

### SUMMARY

This paper presents the normal serum creatinine values in 408 healthy children aged 4-13

All determinations were carried out with a minimum volume of 50  $\mu$ l serum specimen. Mean values, standard deviations and regression lines were calculated in relation to age and body surface for practical reasons of follow up and assessment in individual patients.

### Statistical appendix

Children were classified according to age, body surface area and sex; creatinine levels were studied in relation to this classification (Table I).

It is clear that the influence of sex is small and not significant. Regression lines were therefore calculated for boys and girls together (Table II).

The two regression lines from Figs 1 and 2 can be expressed as follows:

Table 2 Analysis of regression

Source of variation	df	Sum of squares	Mean square	F	P
Total	407	4.522	0.011		
Regression on age	1	1.606	1.606	223.662	0.0000
Error	406	2.915	0.007		
Regression on body surface area	1	1.443	1.443	190.223	0.0000
Error	406	3.079	0.008		
Regression on both	2	1.636	0.818	114.758	0.0000
Error	405	2.886	0.007		

Table I Serum creatinine levels with standard deviations in relationship to age and boy surface area

Age (years)	4	5	6	7
Boys (number)	10	21	27	26
Mean creatinine value	0.362	0.415	0.429	0.439
Standard deviation	0.066	0.076	0.072	0.089
Range	(0.23-0.46)	(0.29-0.63)	(0.32-0.60)	(0.23-0.99)
Girls (number)	16	22	22	34
Mean creatinine value	0.382	0.400	0.432	0.439
Standard deviation	0.058	0.065	0.083	0.097
Range	(0.30-0.48)	(0.28-0.57)	(0.29-0.56)	(0.27-0.88)

Body surface area in square centimetres				
	<6.575	6.575	7.450	8.325
		7.450	8.325	9.200
Boys (number)	6	20	31	17
Mean creatinine value	0.430	0.389	0.417	0.433
Standard deviation	0.129	0.053	0.082	0.078
Range	(0.23-0.63)	(0.23-0.49)	(0.29-0.60)	(0.29-0.99)
Girls (number)	15	22	20	34
Mean creatinine value	0.390	0.384	0.423	0.443
Standard deviation	0.053	0.080	0.077	0.078
Range	(0.30-0.48)	(0.28-0.57)	(0.29-0.61)	(0.27-0.88)

extended periods did not influence serum creatinine (1). Our results are in agreement with those of Kuhlback *et al* (3) and those of Josephson *et al* (2) who made similar studies of smaller numbers of healthy children.

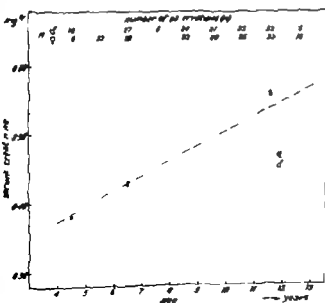


Fig 1 Mean values of serum creatinine in relation to age of 200 healthy boys and 208 healthy girls. Correlation coefficient between creatinine and age = 0.596.

There was no significant difference between the creatinine values for boys and girls with regard to age as well as to body surface area.

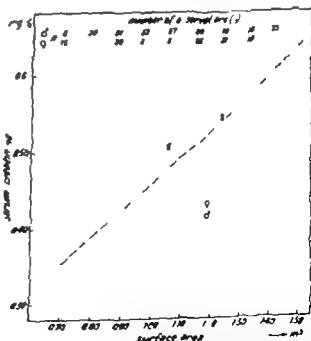


Fig 2 Mean values of serum creatinine in relation to body surface of 200 healthy boys and 208 healthy girls. Correlation coefficient between creatinine and body surface = 0.565.

## IMPAIRED LYMPHOCYTE TRANSFORMATION AND CHROMOSOMAL ABNORMALITIES IN FATAL GRANULOMATOUS DISEASE OF CHILDHOOD

R. D. BARNES, N. P. BISHUN and JEAN HOLLIDAY

From the M.R.C. Clinical Research Centre, Institute of Child Health, University of  
London, London, United Kingdom

In recent years fatal granulomatous disease of childhood has been characterized as an inherited ill disorder that usually presents clinically in male infants with recurrent and persistent infection (3-5) and thus has been associated with an abnormality of leucocyte function (8). Various tests have been described to record a defective intracellular bactericidal action despite normal phagocytosis (8-12). An intracellular nitro-blue tetrazolium (NBT) method first showed the asymptomatic intermediate carrier state in the mothers of such patients and this suggested a sex-linked transmission for the disease (12). This clinical syndrome has now been described in several girls and the fact that the parents have normal leucocyte function suggests that the disease may also be transmitted as an autosomal recessive trait.

More recently a further case of fatal granulomatous disease was described where an abnormal leucocyte bactericidal activity and nitro-blue tetrazolium (NBT) reduction was also detected in the father and upon this a new mode of genetic transmission was proposed (11). This case is again reported here firstly as it presented two new additional interesting features and also since the original abnormality detected in the father of this patient could not be confirmed.

### Case Record

D.B. a 5-year-old boy first presented at the age of 4 months with infection. Since the first admission

the patient has been seen on many occasions with recurrent and persistent infections. The most common site of infection was the respiratory tract. After the age of 14 months when hepatosplenomegaly was first recorded recurrent episodes of osteomyelitis and chronic granulomatous lymphadenitis became the most troublesome sites of persistent infection.

The family history showed that both parents and two siblings a boy aged 8 and a female aged 6 were sick and well. Another sibling a male had died previously with septicaemia at the age of 7 weeks.

### MATERIALS AND METHODS

*In vitro* lymphocyte transformation to phytohemagglutinin (PHA) was performed on peripheral blood. 5 ml of blood was usually taken into a sterile plastic syringe containing 0.5 ml of potassium (phenyl free) heparin. 0.5 ml aliquots of this were then added to 7.0 ml of tissue culture media (6 ml of  $10^{-6}$  by antibiotic buffered 199 solution with 1 ml of freshly activated AB plasma). Penicillin and streptomycin were added to achieve a final concentration of 100 international units (IU) and 74.5 IU/ml respectively. Finally either 0.1 ml of PHA or normal physiological saline (as a control) were added to the cultures prior to incubation.

On occasion the blood cells were first washed three times in an excess of buffered 199 solution prior to the addition of PHA. Again in other cultures 0.5 ml of serum or serum protein fractions were added before the addition of PHA.

All cultures were incubated for 72 hours at 37°C before collection (0.03 ml) was added. After an hour a further incubation blood films were made from the centrifuged pellet of cells with the addition of two drops of heparin serum albumin. These films were subsequently stained with May-Grunwald Giemsa and examined by conventional microscopy. In each preparation 1000 nucleated cells were classified and the numbers of blast forms and cells in mitosis were recorded.

creatinine in mg/100 ml =  $0.254 + 0.026 \times \text{age in years}$   
 creatinine in mg/100 ml =  $0.190 + 0.274 \times \text{body surface area in m}^2$

To conclude the correlation coefficients between the three factors are given

creatinine - age  $-0.596$

creatinine - body surface area  $-0.565$

age - body surface area  $-0.895$

## REFERENCES

- 1 Henry J B Clinical chemistry In I Davidson & J B Henry (eds) *Clinical diagnosis by laboratory methods* W B Saunders New York 1969 14th edn p 545
- 2 Josephson B Fürst H & Järnmark O Age variations in the concentration of non protein nitrogen creatinine and urea in blood of infants and children *Acta Paediat Scand Suppl* 135 111 1962
- 3 Kuhlbroch B Pistorback A Ljungh A & Stenberg M Serum creatinine and creatinine in children and adolescents *Scand J Clin Lab Invest* 22 47 1968

- 4 Sanz M C Ultramicro methods and standardization of equipment *Clin Chem* 3 406 (Suppl) 1957
- 5 Stoop J W Zegers B J M Sander P C & Balheux R E Serum immunoglobulin levels in healthy children and adults *Clin Exp Immun* 4 101 1969
- 6 de Vries L A & van Drietschur J J Bepaling van creatinine in serum volgens Folm In E Gorter & W C de Graaf (eds) *Klinische Diagnostiek* H F Stenfert Kroese NV Leiden 1955 7th edn p 278

Submitted Sept 16 1969

Accepted Dec 18 1969

(R A D)  
 Wilhelmina Kinderziekenhuis  
 Nieuwe Gracht 137  
 Utrecht  
 The Netherlands

**Key words** True creatinine normal values ultra microdetermination renal function

# IMPAIRED LYMPHOCYTE TRANSFORMATION AND CHROMOSOMAL ABNORMALITIES IN FATAL GRANULOMATOUS DISEASE OF CHILDHOOD

R D BARNES N P BISHUN and JEAN HOLLIDAY

From the M.R.C. Clinical Research Centre Institute of Child Health University of London London United Kingdom

In recent years fatal granulomatous disease of childhood has been characterized as an inherited disorder that usually presents clinically in male infants with recurrent and persistent infection (3-5) and this has been associated with an abnormality of leucocyte function (8). Various tests have been described to record a defective intracellular bactericidal action despite normal phagocytosis (8-12). An intracellular nitro-blue tetrazolium (NBT) method first showed the asymptomatic intermediate carrier state in the mothers of such patients and this suggested a sex linked transmission for the disease (12). This clinical syndrome has now been described in several girls and the fact that the parents have normal leucocyte function suggests that the disease may also be transmitted as an autosomal recessive trait.

More recently a further case of fatal granulomatous disease was described where an abnormal leucocyte bactericidal activity and nitro-blue tetrazolium (NBT) reduction was also detected in the father and upon this a new mode of genetic transmission was proposed (11). This case is again reported here firstly as it presented two new additional interesting features and also since the original abnormality detected in the father of this patient could not be confirmed.

## Case Record

D.B. a 3 year-old boy first presented at the age of 4 months with infection. Since this first admission

the patient has been seen on many occasions with recurrent and persistent infections. The most common site of infection was the respiratory tract. After the age of 14 months when hepatosplenomegaly was first recorded recurrent episodes of osteomyelitis and chronic granulomatous lymphadenitis became the most troublesome sites of persistent infection.

The family history showed that both parents and two siblings a boy aged 8 and a female aged 6 were alive and well. Another sibling a male had died previously with septicaemia at the age of 7 weeks.

## MATERIALS AND METHODS

*In vitro* lymphocyte transformation to phytohemagglutinin (PHA) was performed on peripheral blood. 5 ml of blood was initially taken into a sterile plastic syringe containing 0.5 ml of non-toxic (phenol free) heparin. 0.5 ml aliquots of this were then added to 7.0 ml of tissue culture media (6 ml of 10% foetal bovine serum buffered 199\* solution + 2 ml of freshly activated AB plasma). Penicillin and streptomycin were added to achieve a final concentration of 100 international units (IU) and 745 IU/ml respectively. Finally either 0.1 ml of PHA or normal physiological saline (as a control) were added to the cultures prior to incubation.

On occasion the blood cells were first washed three times in an excess of buffered 199 solution prior to the addition of PHA. Again in other cultures 0.5 ml of serum or serum protein fractions were added before the addition of PHA.

All cultures were incubated for 72 hours at 37°C before colchicine (0.03 µl) was added. After an hour's further incubation blood films were made from the centrifuged pellet of cells with the addition of two drops of bovine serum albumin. These films were subsequently stained with May-Grimwald-Giemsa and examined by conventional microscopy. In each preparation 1000 nucleated cells were classified and the numbers of blast forms and cells in mitosis were recorded.



creatinine in mg/100 ml =  $0.254 + 0.026 \times \text{age in years}$   
 creatinine in mg/100 ml =  $0.190 + 0.274 \times \text{body surface area in m}$

To conclude the correlation coefficients between the three factors are given

creatinine - age = 0.596

creatinine - body surface area = 0.565

age - body surface area = 0.885

## REFERENCES

- 1 Henry J B Clinical chemistry In I Davidson & J B Henry (eds) *Clinical diagnosis by laboratory methods* W B Saunders New York 1969 14th edn p 545
- 2 Josephson B, Furst P & Jannmark O Age variations in the concentration of non protein nitrogen, creatinine and urea in blood of infants and children *Acta Paediat Scand Suppl* 135 111 1962
- 3 Kuhlback B, Pisterneck A, Luoma H & Stenberg M Serum creatine and creatinine in children and adolescents *Scand J Clin Lab Invest* 22 47 1968

- 4 Sanz M C Ultramicro methods and standardization of equipment *Clin Chem* 3 406 (Suppl) 1957
- 5 Stoop J W, Zegers B J M, Sander F C & Ballieux R E Serum immunoglobulin levels in healthy children and adults *Clin Exp Immun* 4 101 1969
- 6 de Vries L A & van Daetselaar J J Bepaling van creatinine in serum volgens Folm In E Gorter & W C de Graaf (eds) *Altrische Diagnostiek* H E Stenfert Kroese NV Leiden 1955 7th edn p 278

Submitted Sept 16 1969

Accepted Dec 18 1969

(R A D)  
 Wilhelmina Kinderziekenhuis  
 Nieuwe Gracht 137  
 Utrecht  
 The Netherlands

**Key words:** True creatinine normal values ultra microdetermination renal function



Fig 1 Serum inhibitor to *in vitro* lymphocyte transformation

no numerical chromosome abnormalities in the proband although in 20 cells (5%) structural abnormalities as of the kind shown in Fig. 4 were observed. These were not restricted to one particular chromosome or group of chromosomes.

The abnormalities obtained on both occasions from the mother's cultures were in certain respects similar to those obtained in the proband (as in Fig. 4). Polyploidy and hyperdiploidy were found in a small proportion of her cells but structural abnormalities were obtained in 40% of the cells examined on the prior occasion (Figs 5 and 6), the majority of which were diploid on both occasions. The cells containing 45 chromosomes could have been formed from cell breakage during the preparation of the slides since different chromosomes were missing in each cell examined.

The chromosomes of the proband's father were normal in all respects.

### DISCUSSION

This case of fatal granulomatous disease of childhood presented the characteristic clinical

pattern of recurrent and persistent infection with hepatosplenomegaly and the grossly abnormal result with leucocyte phagocytosis and digestion test of Holmes *et al* (8) confirmed this diagnosis. The fact that the patient's mother presents an intermediate response in this test would suggest that this patient is very similar to the previously described sex-linked cases (6). In this respect the normal results obtained for the father would support this. The limitation of the techniques used by Thompson and her co-workers (11) to demonstrate an apparent abnormality in the father on two different occasions have been stressed by others (13) (7) and this could account for the contrasting results. There is an alternative explanation possible. Alexander *et al* (1) noted a transient leucocyte dysfunction in several different disorders and it is conceivable that it is this which has been detected by Thompson *et al* (11). Meanwhile at this stage it seems unwise to modify any hypothesis concerning the genetic transmission of fatal granulomatous disease until either these contrasting results in

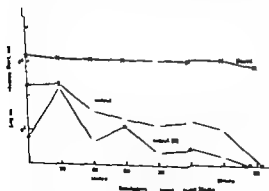


Fig. 2 Comparison of the destruction of *S. aureus* by peripheral white cells obtained from two controls and the patient with fatal granulomatous disease.

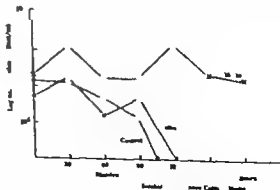


Fig. 3 Comparison of the destruction of *S. aureus* by peripheral white cells obtained from a control and the mother and father of a patient with fatal granulomatous disease.

Table 1 *In vitro* lymphocyte transformation to PHA

Source of lymphocytes	of nucleated cells	
	Blast forms	Mitoses
Patient	46	22
Controls 1	570	30
2	510	100
3	502	12
4	550	36
5	456	16

Table 2 *The effect of washing the cells prior to stimulation with PHA*

Treatment	of nucleated cells	
	Blast forms	Mitoses
Patient Unwashed	156	08
Washed and resuspended in normal control (1) sera	336	22
Washed and resuspended in normal control (2) sera	470	42

Serum fractionation was initially performed with the addition of an equal volume of saturated ammonium sulphate solution. The crude globulin precipitate resuspended in a small volume of normal saline was dialysed free of sulphate an aliquot retained (F) whilst the remainder was further fractionated through DEAE cellulose into three fractions (F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>) according to the method of Peterson & Sober (10). The original soluble serum fraction remaining after precipitation with the saturated ammonium sulphate was also dialysed free of sulphate and known as the crude albumin fraction (F<sub>4</sub>). All fractions were finally dialysed against large volumes of normal physiological saline and sterilized by passage through a Millipore 0.25 filter prior to use. Immunoelectrophoresis with goat anti human serum (Hyland Laboratories, Los Angeles) was used to confirm the nature of the fractions.

*Polymorph phagocytosis test* was performed according to the method of Holmes et al (8).

*Cytogenetic examination* was performed on 100 cytologically suitable cells obtained from peripheral blood as previously described by Bahun et al (4).

## RESULTS

At 14 months of age when the child presented with recurrent infected ischiorectal fistulae and bilateral suppurative inguinal adenitis

*In vitro* lymphocyte transformation test in the patient showed a marked reduction in the number of "blast" forms when compared with five normal controls (Table 1).

At 18 months of age when the patient presented with osteomyelitis of the right olecranon and right tibia

*In vitro* lymphocyte transformation was again impaired however a relatively high transformation index was obtained (% of blast cells) when the cells were washed (Table 2).

The transformation of normal lymphocytes from red cell compatible control samples was impaired on the addition of the patient's serum and this was later shown to be associated with the crude globulin F<sub>1</sub> and F<sub>2</sub> serum fractions (Table 3) the latter was shown by immunoelectrophoresis to be largely IgG (Fig 1).

At 4 years of age the infant was readmitted with recurrent and persistent infections and

(a) *The polymorph phagocytosis test* showed that the patient, compared with controls had normal phagocytosis but a diminished ability to digest bacteria (Fig 2). The mother was noted to have an intermediate level whilst the two other siblings and the father were normal in this test (Fig 3).

(b) *In vitro lymphocyte transformation to PHA* was normal (transformation index 73%).

(c) *Chromosome studies* on peripheral blood from the patient, his mother (two observations) and father are shown in Table 4. There were

Table 3 *The inhibition of normal lymphocyte transformation with serum fractions from the patient*

Source of lymphocytes and serum fractions used	of nucleated cells	
	Blast forms	Mitoses
Normal (control) peripheral blood (washed)		
Patient's whole serum	136	08
Patient's F <sub>1</sub>	346	10
Patient's F <sub>2</sub>	126	08
Patient's F <sub>3</sub>	108	04
Patient's F <sub>4</sub>	238	10
Patient's F <sub>5</sub>	230	12



Fig 1 Serum inhibitor to *in vitro* lymphocyte transformation

no numerical chromosome abnormalities in the proband although in 20 cells (5%) structural abnormalities as of the kind shown in Fig 4 were observed. These were not restricted to one particular chromosome or group of chromosomes.

The abnormalities obtained on both occasions from the mother's cultures were in certain respects similar to those obtained in the proband (as in Fig 4). Polyploidy and hyperdiploidy were found in a small proportion of her cells but structural abnormalities were obtained in 40% of the cells examined on the prior occasion (Figs 5 and 6) the majority of which were diploid on both occasions. The cells containing 45 chromosomes could have been formed from cell breakage during the preparation of the slides since different chromosomes were missing in each cell examined.

The chromosomes of the proband's father were normal in all respects.

### DISCUSSION

This case of fatal granulomatous disease of childhood presented the characteristic clinical

pattern of recurrent and persistent infection with hepatosplenomegaly and the grossly abnormal result with leucocyte phagocytosis and digestion test of Holmes *et al* (8) confirmed this diagnosis. The fact that the patient's mother presents an "intermediate response" in this test would suggest that this patient is very similar to the previously described sex-linked cases (6). In this respect the normal results obtained for the father would support this. The limitation of the techniques used by Thompson and her co-workers (11) to demonstrate an apparent abnormality in the father on two different occasions have been stressed by others (13) (7) and this could account for the contrasting results. There is an alternative explanation possible. Alexander *et al* (1) noted a transient leucocyte dysfunction in several different disorders and it is conceivable that it is this which has been detected by Thompson *et al* (11). Meanwhile at this stage it seems unwise to modify any hypothesis concerning the genetic transmission of fatal granulomatous disease until either these contrasting results in

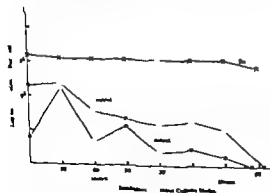


Fig 2 Comparison of the destruction of *S. aureus* by peripheral white cells obtained from two controls and the patient with fatal granulomatous disease

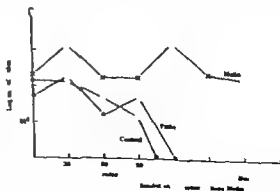


Fig 3 Comparison of the destruction of *S. aureus* by peripheral white cells obtained from a control and the mother and father of a patient with fatal granulomatous disease

Table 4 Chromosomal pattern in the peripheral blood of the parents and the patient with fatal granulomatous disease

Subject	No. of cells karyotyped	Chromosome counts						Total cells	Comment
		<44	45	46	47	>48	Poly		
Propositus	10	1	0	97	—	—	2	100	5 Abnormal Cells
Mother (1)	20	—	5	89	—	2	4	100	40 Abnormal Cells
(2)	5	1	3	91	—	—	5	100	20 Abnormal Cells
Father	3	1	1	97	—	—	1	100	Normal

the father of this patient are explained or other similar cases described

The impaired lymphocyte transformation to PHA originally noted was very surprising particularly when traced to the presence of a serum factor. The transient nature of this was suggested when the *in vitro* response became normal when tested at 4 years of age. The significance of this remains unknown however it is important to note that serum inhibitors to *in vitro* lymphocyte transformation exist in this and other conditions (9) and that an apparent impaired transformation does not necessarily primarily implicate the lymphocyte. Good *et al* (6) in their review of cases with fatal granulomatous disease noted lymphocyte transformation to be normal and the case here is the first to be recorded where transformation was impaired.

This case is also the first description of abnormal chromosomes in fatal granulomatous disease of childhood. The significance of the abnormality and the incidence of such changes in this disease however, remain speculative. Chromosomal abnormalities are essentially congenital or acquired and until further cases are studied the cause of the abnormalities here must be considered unknown. If the chromosomal abnormalities in both the propositus and his mother are congenital this might be considered, in the absence of any abnormal cytogenetic findings in the father that the mode of transmission of this might be sex linked. This in turn might be indirect evidence to favour a similar sex linked transmission of fatal granulomatous disease of childhood in this child. Of course the chromosomal abnormalities in both the mother and the affected offspring may have

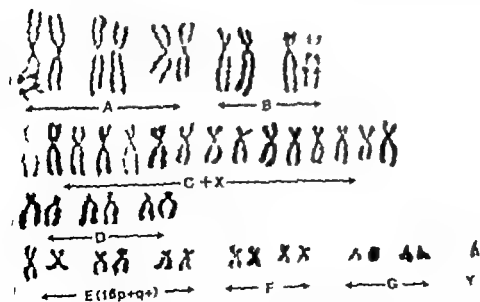


Fig. 4 Karyotype of a cell from propositus showing enlargement of both arms of no. 16 pair of chromosomes (not a constant feature in all cells). Two of the cells karyotyped from the mother also showed this feature.



Fig 5 Karyotype of a cell from mother showing chromosomal break (asterisk) and enlargement of long arm of a D chromosome

been acquired independently and this view is probably favoured by the fact that the abnormalities were different. If these chromosomal abnormalities had been acquired it would be interesting to know the cause. The patient at the time of investigation was suffering from inevitable infection but the mother on both

occasions when tested was apparently clinically normal and no infection was obvious. It is conceivable that chromosomal abnormalities are a feature of both fatal granulomatous disease and its carrier state and if so might be a valuable diagnostic adjunct if further studies can confirm this.



Fig 6 Karyotype of another cell from mother showing chromosomal break (asterisk) and ill-defined chromosome

Table 4 Chromosomal pattern in the peripheral blood of the parents and the patient with fatal granulomatous disease

Subject	No. of cells karyotyped	Chromosome counts						Total cells	Comment
		<44	45	46	47	>48	Poly		
Propositus	10	1	0	97	—	—	2	100	5 Abnormal Cells
Mother (1)	20	—	5	89	—	2	4	100	40 Abnormal Cells
(2)	5	1	3	91	—	—	5	100	20 Abnormal Cells
Father	3	1	1	97	—	—	1	100	Normal

the father of this patient are explained or other similar cases described.

The impaired lymphocyte transformation to PHA originally noted was very surprising particularly when traced to the presence of a serum factor. The transient nature of this was suggested when the *in vitro* response became normal when tested at 4 years of age. The significance of this remains unknown however it is important to note that serum inhibitors to *in vitro* lymphocyte transformation exist in this and other conditions (9) and that an apparent unpaired transformation does not necessarily primarily implicate the lymphocyte. Good *et al* (6) in their review of cases with fatal granulomatous disease noted lymphocyte transformation to be normal and the case here is the first to be recorded where transformation was impaired.

This case is also the first description of abnormal chromosomes in fatal granulomatous disease of childhood. The significance of the abnormality and the incidence of such changes in this disease, however remain speculative. Chromosomal abnormalities are essentially congenital or acquired and until further cases are studied the cause of the abnormalities here must be considered unknown. If the chromosomal abnormalities in both the propositus and his mother are congenital this might be considered in the absence of any abnormal cytogenetic findings in the father that the mode of transmission of this might be sex linked. This in turn might be indirect evidence to favour a similar sex linked transmission of fatal granulomatous disease of childhood in this child. Of course the chromosomal abnormalities in both the mother and the affected offspring may have

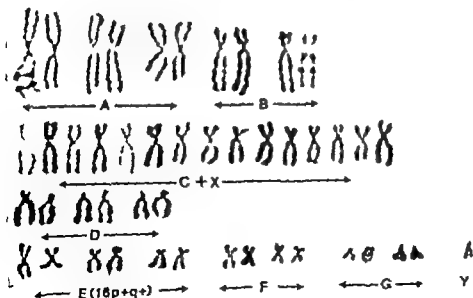


Fig. 4 Karyotype of a cell from propositus showing enlargement of both arms of no. 16 pair of chromosomes (not a constant feature in all cells). Two of the cells karyotyped from the mother also showed this feature.

## THE USE OF AZATHIOPRINE IN REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDREN

MARGARET W. HILGARTNER, PHILIP LANZKOWSKY and CARL H. SMITH

From the Division of Pediatric Hematology, New York Hospital—Cornell University Medical College, New York, U.S.A.

Corticosteroids and splenectomy are accepted therapeutic measures for treatment of Idiopathic Thrombocytopenic Purpura (ITP). Boursic & Doan (3) reported 77 of 273 patients refractory to both modes of therapy and that 44 died from fatal bleeding. These methods of treatment are not ideal since prolonged steroid therapy has undesirable and occasionally dangerous complications and splenectomy in children might be associated with life threatening and occasionally fatal infections (12). A logical extension of the concept that ITP is an autoimmune disorder (2) is to attempt to treat it by drugs capable of suppressing the immune response (1). Since azathioprine (Imuran) is an effective immunosuppressive agent, is easily controlled and the least toxic of the immunosuppressive drugs, its effectiveness in refractory ITP in children merits trial. Preliminary results of the use of this drug, predominantly in adults with ITP, have been very encouraging (3, 4 & 13). The purpose of this paper is to report our findings in 3 children with refractory ITP treated with azathioprine.

### MATERIALS AND METHODS

Five children (3 males and 2 females) with chronic ITP aged from 2 years to 11 years were evalua-

This work was supported by the Children's Blood Foundation, Inc. Azathioprine (Imuran) Bournoughs Wellcome & Co. (U.S.A.) Inc.

ated for the results of therapy with azathioprine. Of the patients 2 had had splenectomy and required steroids (1.2 mg/kg) post splenectomy for 3 to 36 months respectively for control of bleeding and 3 non splenectomized children required persistent steroid therapy to control symptoms.

The following criteria were used for selection of patients to receive azathioprine therapy:

1. A diagnosis of idiopathic thrombocytopenic purpura with bone marrow findings consistent with this diagnosis. Patient with secondary thrombocytopenic purpura were not included.

2. The patient had been treated with steroids in a dose of 20 mg/kg/day for 4-6 weeks on at least two occasions and had relapsed requiring either maintenance steroid therapy to control bleeding with a platelet count below 40,000/mm<sup>3</sup> or had relapsed post-splenectomy and required steroid therapy to control symptoms with platelets between 10-40,000/mm<sup>3</sup>.

Before azathioprine therapy was started the patients were closely supervised without steroid therapy for at least 4 weeks to obviate the possible influence of prolonged corticosteroid therapy and remission of thrombocytopenia with its cessation (5). The following basic lab. tests were carried out: liver function studies (total serum protein, albumin, globulin, alkaline phosphatase, bilirubin and SGOT), Coombs test, hepatic preparations and test for platelet antibodies. After this observation period azathioprine in a dose of 2.0 mg/kg/day was given. If there was no platelet response within 4 weeks the dose was increased to 3-4 mg/kg/day and if there was no response in 8 weeks a small dose of steroids (0.2 mg/kg/day) was added. Complete blood count was carried out twice weekly for 5 weeks and thereafter every 2-3 weeks depending on the degree of leukopenia and platelet count.

We were prepared to permit the leukocyte count to drop to 4,000/mm<sup>3</sup> and the hemoglobin level to drop 2 g per 100 ml before decreasing the dosage of the drug. However this degree of hematologic depression



## SUMMARY

A previously described case of fatal granulomatous disease of childhood is presented here as certain previous findings could not be confirmed and since two unusual features were noted. Upon the basis of impaired leucocyte function in the father of this patient a new mode of genetic transmission for the disease was proposed meanwhile we have been unable to confirm these original findings (11). This case in itself was interesting since firstly 'in impaired *in vitro* lymphocyte transformation was noted and this later related to the presence of a serum inhibitory factor. Secondly chromosomal abnormalities were recorded in peripheral blood cells of both patient and his mother and this might be considered evidence to favour a sex linked transmission of the disease in this case.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance of Mrs J Thomas.

The work was supported by the Joint Research Board of the Institute of Child Health and Hospital for Sick Children together with grants from the Camille Samuel Trust and the Wellcome Trust.

## REFERENCES

- Alexander J W, Windhorst D B & Good R A. Improved tests for the evaluation of neutrophil function in human disease. *J Lab clin Med* 72 136 1968.
- Baehner R L & Nathan D G. Quantitative nitroblue tetrazolium test in chronic granulomatous disease. *New Eng J Med* 278 971 1968.
- Berendes H, Bridges R A & Good R A. A fatal granulomatous disease of childhood: the clinical study of a new syndrome. *Ann Med* 40 309 1957.
- Bahan N P, Morton W R M & McLaverty H. Macromethod for culturing leucocytes for chromosomal analysis. *Lancet* II 315 1964.
- Bridges R A, Berendes H & Good R A. A fatal granulomatous disease of childhood: the clinical, pathological and laboratory features of a new syndrome. *Amer J Dis Child* 97 387 1959.
- Good R A, Que P G, Windhorst D B, Page A R, Rodey G E, White J, Wolfson J J & Holmes B H. Fatal (chronic) granulomatous disease of childhood: A hereditary defect of leucocyte function. *Seminars in Hematology* v 215 1968.
- Gruhn O C & Mauer A M. Neutrophil Function and NBT Dye Reduction. *Lancet* II 383 1969.
- Holmes B, Que P G, Windhorst D B, Pollara B & Good R A. Fatal granulomatous disease of childhood: An abnormality of phagocytic function. *Lancet* I 1227 1966.
- Levene G M, Turk J L, Wraith D J M & Grimbale A G S. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. *Lancet* II 246 1969.
- Peterson E A & Sober H A. In S P Coldwick & N O Kaplan (eds) *Methods in Enzymology*. Academic Press, New York 1962 vol 2 p 3.
- Thompson E N, Chandra R K, Cope W A & Soothill J F. Leucocyte abnormality in both parents of a patient with chronic granulomatous disease. *Lancet* I 799 1969.
- Windhorst D B, Holmes B & Good R A. A newly defined X linked trait in man with demonstration of the Lyon effect in carrier females. *Lancet* I 737 1967.
- Windhorst D B. Inheritance of fatal granulomatous disease. *Lancet* II 543 1969.

Submitted Oct 27 1969

Accepted Jan 10 1970

(RDB)

MRC Clinical Research Centre  
Institute of Child Health  
30 Guilford Street  
London WC1  
United Kingdom

**Key words** Chronic granulomatous disease of childhood; chromosomal abnormalities; lymphocyte transformation inhibition.

*Case 4 (E. V. - NYH no 106-46-80)*

A 27 month-old girl developed purpura. The bone marrow findings were consistent with idiopathic thrombocytopenic purpura. She was treated with Prednisone 0.5 mg/kg/day for 6 months without response then taken off all therapy for 7 months. Because of bruising and epistaxis a splenectomy was performed 13 months after the onset of the disease with good platelet response to 500 000/mm<sup>3</sup>. She was well for 15 months post splenectomy when purpura and epistaxis reappeared. Steroid therapy 2 mg/kg/day was given for 12 weeks and tapered without rise of platelets over 30 000/mm<sup>3</sup>. After 10 weeks off therapy azathioprine 2.5 mg/kg/day was given for 3 months without response. Prednisone was added at 0.2 mg/day. She responded slowly over 8 months. Steroids were tapered over 4 months and azathioprine over an additional 8 month period. Platelets fell with upper respiratory infections but returned to normal thereafter and remained so eight months after therapy was stopped. The results of 2 years of therapy are excellent (Fig. 4).

*Case 5 (R. A. - NYH no 78-54-27)*

In 1956 this boy developed easy bruising and severe epistaxis at 2 1/2 years of age. A bone marrow examination was compatible with ITP. Platelet count was less than 10 000/mm<sup>3</sup>. He was treated with Prednisone 1 mg/kg/day for three years with no improvement. The dose was increased to 2 mg/kg/day over the next year with some improvement but relapse with bruising and petechiae occurred when therapy was decreased and discontinued. A third course of steroids 2 mg/kg/day produced a rise in platelet count to 100 000/mm<sup>3</sup> with subsequent fall when therapy was discontinued. Splenectomy was performed in May 1961 with good results. Platelets rose to 410 000/mm<sup>3</sup> post splenectomy. The patient had a relapse 2 1/2 years later with petechiae and gastro-intestinal bleeding; platelet count was 34 000/

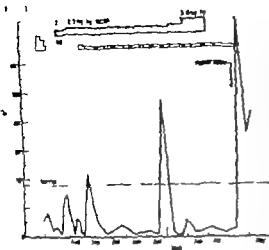


Fig. 3 Course of disease in case 3 (5 D B D 1/62)

mm<sup>3</sup> 1 mg/kg/day for four months with good response followed by four months without therapy when she was well. Petechiae returned and she was given Prednisone 2 mg/kg/day with a good response. After 6 weeks steroids were tapered and petechiae and bruising returned. She was maintained without steroid therapy for 4 weeks. Azathioprine 2.5 mg/kg/day was given for six weeks with poor response. Steroids at 1 mg/kg/day were added to the regimen without sustained response. Azathioprine was increased to 4.0 mg/kg/day for an additional month when a raised SGOT was observed and the drug was discontinued. Since there had been no response for an 8 month period this patient was considered a failure to azathioprine therapy and splenectomy was performed with excellent results (Fig. 3).

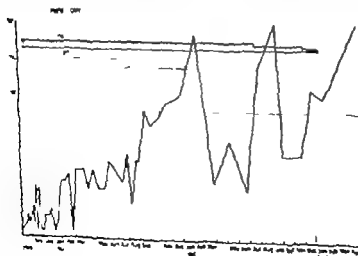


Fig. 4 Course of disease in case 4 (E. V. B D 10 11 Splenectomy 2/55)

FIGURE 1

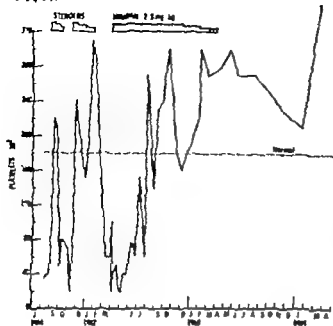


Fig 1 Course of disease in case 1 (P B B D 8/54)

were not observed. Therapy was continued for 8 months before considered a failure and in successful cases was continued for at least 2 months after a satisfactory platelet count was obtained before the drugs were tapered. Immunophoretic studies were done from time to time during therapy.

## CASE REPORTS

### Case 1 (P B - NYH no 106 22 93)

A 12 year old boy had sudden onset of petechiae and bruising. The bone marrow findings were consistent with ITP. He was treated with Prednisone 2 mg/kg/day for six weeks with rise of platelets to 220 000/

mm<sup>3</sup> but relapsed with petechiae and ecchymosis and platelets fell to 20 000/mm<sup>3</sup> when therapy was discontinued. A second course of steroids in the same dose given for 10 weeks was followed by a rise of platelets to 310 100/mm<sup>3</sup> but when the steroids were discontinued relapse occurred again. Eight months after onset of symptoms azathioprine 2.5 mg/kg/day was begun. He had a slow response over a 4 month period with platelet count rising to 270 000/mm<sup>3</sup>. He was maintained for additional 4 months on the same dosage and then tapered slowly over 4 months. Total duration of azathioprine therapy was 12 months. The result of therapy was excellent (Fig 1).

### Case 2 (C C - NYH no 107 86 99)

A 10 1/2-year old girl had gradual onset of petechiae and ecchymosis. The bone marrow aspiration was consistent with ITP. She was placed on Prednisone at 1 mg/kg/day with good results. Platelet count maintained high at 220 000/mm<sup>3</sup> as steroids were withdrawn. Two weeks after discontinuing steroids the patient developed mumps with a return of ecchymosis and petechiae in 1 week with platelet count of 10 000/mm<sup>3</sup>. Steroids in the dose of 2 mg/kg/day again produced a good response and platelet count rose to 280 000/mm<sup>3</sup>. She was well for seven months without any treatment when symptoms returned and azathioprine was begun at 2.0 mg/kg/day. She had a slow response over a 7 month period possibly accelerated by a rise in dosage to 3.0 mg/kg. Total duration of therapy on azathioprine was 12 months. She has remained well and asymptomatic 7 months after azathioprine was stopped. The results are excellent (Fig 2).

### Case 3 (S D - NYH no 109 20 29)

A 4 1/2-year old girl had a sudden onset of petechiae and severe ecchymosis and the bone marrow findings were consistent with ITP. She was treated with Pred

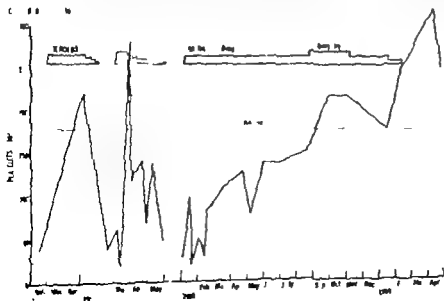


Fig 2 Course of disease in case 2 (C C C B D 3/56)

Table 2 Toxicity observed during azathioprine therapy

Case no	Hemoglobin levels g/100 ml			Leukocyte levels /mm <sup>3</sup>			Liver function tests		
	Initial	Lowest	Present	Initial	Lowest	Present	Initial	During therapy	Present
1 P B	13.8	11.4	15.1	8 950	2 900	5 000	Normal	No change	Normal
2 C C	12.4	11.4	13.0	7 850	3 100	8 200	Normal	No change	Normal
3 S D	11.7	10.3	11.4	10 700	7 700	8 450	Normal	SGOT 55 (nl 20-40 U/ml) SGPT 11.9 (nl 5-40 U/ml)	Normal
4 E V	1.5	10.6	11.7	8 900	3 150	6 600	Normal	No change	Normal
5 R K	14.8	13.0	14.5	6 100	3 100	10 600	Normal	No change	Normal

mission without relapse after discontinuation of all therapy 2) Good excellent initial response 3) Relapsed requiring treatment or 4) Failure no response

An excellent result was obtained in 3 patients a good result in 1 and 1 patient failed to respond. Of the patients who had responded 2 had not undergone splenectomy and 2 had relapsed post splenectomy. The 2 post splenectomy patients required low dosage steroids for a temporary period in addition to azathioprine. The one pre splenectomy patient who failed to respond to azathioprine responded well to splenectomy.

Table 2 summarizes toxicity observed during therapy. Slight fall in hemoglobin was observed leukopenia occurred in 4 patients and minimal changes in SGOT occurred in only 1 patient. All these changes were transient.

Platelet antibodies were not found in any of the patients before therapy. One patient had a transiently positive Coombs test which reverted to normal before azathioprine therapy.

Immunophoretic studies done at the end of azathioprine therapy in cases 2, 4 and 5 revealed normal levels of IgG. IgG was 1480 mg/100 ml in case 2, 1300 mg/100 ml in case 4 and 5 with a normal range of 776-1647 mg/100 ml for the age. IgA was at the upper limit of normal with values of 250, 250 and 760 mg/100 ml respectively for cases 2, 4 and 5 with a normal range of 64-290 mg/100 ml. IgM was normal in cases 2 and 4 with levels of 102 mg/100 ml and 106 mg/100 ml and slightly low in case 5, 51 mg/100 ml with

60-140 mg/100 ml the range of normal. After six weeks off therapy this level rose to 70 mg/100 ml.

## DISCUSSION

ITP in childhood is not necessarily a self limited disease of short duration. Many cases become chronic and relapses are known to occur. Although the etiology remains obscure many authors believe it to be an autoimmune phenomenon with spontaneous remission unlikely in those who become chronic.

Since conventional therapy with corticosteroids and splenectomy have significant risks an alternate mode of therapy has been sought. A great deal of interest has been aroused in the suppression of autoimmune disease by antineoplastic agents. Schwartz & Dameshek (11) first showed that mercaptopurine and thioguanine were useful in the treatment of autoimmune hemolytic anemia. Since then these antineoplastic agents have been successfully used to treat other suspected immune diseases such as systemic lupus nephrotic syndrome, rheumatoid arthritis and ulcerative colitis (6, 8, 15). In addition they have been used to suppress antibody formation at the time of tissue transplantation.

More recently azathioprine has been used with increasing frequency because it has less toxicity, less deleterious effect on the bone marrow and greater immunosuppressive effect although it is metabolized to its analogue mercaptopurine in the body (10). It has also been

R K B D 12/53 Splenectomy 5/61

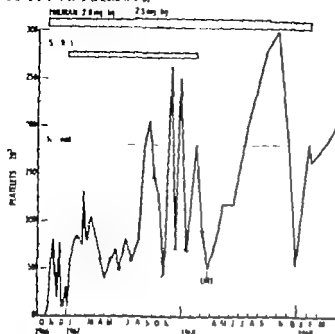


Fig 5 Course of disease in case 5 (R K B D 12/53 Splenectomy 5/61)

mm<sup>3</sup>. A Coombs test was weakly positive at this time but became negative within 3 months and has remained negative. Fresh frozen plasma 30 mg/kg did not produce a platelet rise. Platelet transfusions were given and Prednisone was restarted at 2 mg/kg/day with a rise in platelet count to 170 000/mm<sup>3</sup>. He remained on low dose steroids (0.2 mg/kg/day) for one year without symptoms. Platelet count varied but remained consistently well below normal. In October 1966 it was 10 000/mm<sup>3</sup>. All steroids were discontinued for 4 weeks and in November 1966 azathioprine alone was begun at 2 mg/kg/day. There was little response over a 10 week period. Prednisone was restarted and maintained at 0.1 mg/kg/day with a response seen over the next 6 months. Steroids were discontinued. Platelet count fell with an upper respiratory infection but returned to good levels. When azathioprine was discontinued after total 2 years therapy the platelet count promptly fell to 56 000 with an upper respiratory infection but without return of symptoms then rose to 180 000 held at 200 000/mm<sup>3</sup> for 4 months and fell again to 36 000. He has been restarted on azathioprine 2.5 mg/kg/day and after 2 months of therapy his platelet count is 150 000/mm<sup>3</sup>. Although his course has been long and variable the response to therapy has been designated as good (Fig 5).

## RESULTS

Table 1 summarizes the results of therapy. The response to therapy is graded according to the platelet count and symptomatology as 1) Excellent, complete hematological and clinical re-

Table 1 Azathioprine (murban) therapy for refractory idiopathic thrombocytopenic purpura in childhood

Case no	Sex	Age in yrs at onset	Duration of disease	Previous therapy	Platelet count/mm <sup>3</sup>		Azathioprine dose mg/kg/day	Duration of azathioprine therapy (months)	Concomitant therapy	Duration of remission (mo)	Current therapy	Result
					Initial	Current						
1 P B	M	12	24 mo	Prednisone 2 mg/kg	40 000	220 000	2.5 mg	12	None	12	None	Excellent
2 C C	F	10½	23 mo	Prednisone 2 mg/kg	40 000	220 000	2.0-3.0 mg	12	None	3	None	Excellent
3 S D	F	4½	17 mo	Prednisone 2 mg/kg	50 000	250 000	2.0-5.0 mg	8	Splenectomy	12	None	Failure
4 E V	F	2½	5 yrs	Prednisone 2 mg/kg	10 000	210 000	2.5 mg	24	Prednisolone 0.2 mg/kg	3	None	Excellent
5 R K	M	2½	13½ yrs	Splenectomy Prednisone 2 mg/kg	< 10 000	200 000	2.5 mg	27	Prednisolone 0.2 mg/kg	4	Azathioprine 2.5 mg/kg/day	Good

Table 2 Toxicity observed during azathioprine therapy

Case no.	Hemoglobin levels g/100 ml			Leukocyte levels /mm <sup>3</sup>			Liver function tests		
	Initial	Lowest	Present	Initial	Lowest	Present	Initial	During therapy	Present
1 P R	13.8	11.4	15.1	8 940	2 900	5 000	Normal	No change	Normal
2 C C	12.4	11.4	13.0	7 840	3 100	8 200	Normal	No change	Normal
3 S D	11.7	10.3	11.4	10 700	7 700	8 450	Normal	SGOT 55 (nl 0-40 U/ml) SGPT 119 (nl 5-40 U/ml)	Normal Normal
4 E V	12.5	10.6	11.7	8 900	3 150	6 600	Normal	No change	Normal
5 R K	14.8	13.0	14.5	6 100	3 100	10 600	Normal	No change	Normal

remission without relapse after discontinuation of all therapy 2) Good excellent initial response 3) Relapsed requiring treatment or 4) Failure no response

An excellent result was obtained in 3 patients a good result in 1 and 1 patient failed to respond. Of the patients who had responded 2 had not undergone splenectomy and 2 had relapsed post splenectomy. The 2 post splenectomy patients required low dosage steroids for a temporary period in addition to azathioprine. The one pre splenectomy patient who failed to respond to azathioprine responded well to splenectomy.

Table 2 summarizes toxicity observed during therapy. Slight fall in hemoglobin was observed leukopenia occurred in 4 patients and minimal changes in SGOT occurred in only 1 patient. All these changes were transient.

Platelet antibodies were not found in any of the patients before therapy. One patient had a transiently positive Coombs test which reverted to normal before azathioprine therapy.

Immunophoretic studies done at the end of azathioprine therapy in cases 2, 4 and 5 revealed normal levels of IgG. IgG was 1480 mg/100 ml in case 2, 1300 mg/100 ml in cases 4 and 5 with a normal range of 776-1647 mg/100 ml for the age. IgA was at the upper limit of normal with values of 250, 250 and 260 mg/100 ml respectively for cases 2, 4 and 5 with a normal range of 64-290 mg/100 ml. IgM was normal in cases 2 and 4 with levels of 102 mg/100 ml and 106 mg/100 ml and slightly low in case 5 51 mg/100 ml with

60-140 mg/100 ml the range of normal. After six weeks off therapy this level rose to 70 mg/100 ml.

## DISCUSSION

TTP in childhood is not necessarily a self limited disease of short duration. Many cases become chronic and relapses are known to occur. Although the etiology remains obscure many authors believe it to be an autoimmune phenomenon with spontaneous remission unlikely in those who become chronic.

Since conventional therapy with corticosteroids and splenectomy have significant risks an alternate mode of therapy has been sought. A great deal of interest has been aroused in the suppression of autoimmune disease by antineoplastic agents. Schwartz & Dameshek (11) first showed that mercaptopurine and thioguanine were useful in the treatment of autoimmune hemolytic anemia. Since then these antineoplastic agents have been successfully used to treat other suspected immune diseases such as systemic lupus nephrotic syndrome, rheumatoid arthritis and ulcerative colitis (6, 8, 15). In addition they have been used to suppress antibody formation at the time of tissue transplantation.

More recently azathioprine has been used with increasing frequency because it has less toxicity, less deleterious effect on the bone marrow and greater immunosuppressive effect although it is metabolized to its analogue mercaptopurine in the body (10). It has also been

P. K. 12 4 11 SPLENECTOMY 5/61

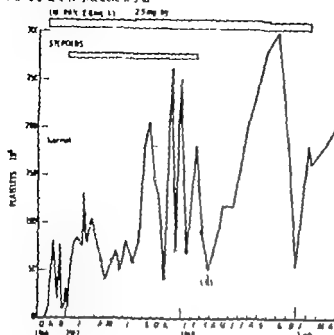


Fig 5 Course of disease in case 5 (R. K. B. D. 12/53 Splenectomy 5/61)

mm. A Coombs test was weakly positive at this time but became negative within 3 months and has remained negative. Fresh frozen plasma 30 mg/kg did not produce a platelet rise. Platelet transfusions were given and Prednisone was restarted at 2 mg/kg/day with a rise in platelet count to 170 000/mm. He remained on low dose steroids (0.2 mg/kg/day) for one year without symptoms. Platelet count varied but remained consistently well below normal. In October 1966 it was 10 000/mm. All steroids were discontinued for 4 weeks and in November 1966 azathioprine alone was begun at 2 mg/kg/day. There was little response over a 10 week period. Prednisone was restarted and maintained at 0.1 mg/kg/day with a response seen over the next 6 months. Steroids were discontinued. Platelet count fell with an upper respiratory infection but returned to good levels. When azathioprine was discontinued after total 2 years therapy the platelet count promptly fell to 36 000 with an upper respiratory infection but without return of symptoms then rose to 180 000 held at 200 000/mm for 4 months and fell again to 36 000. He has been restarted on azathioprine 2.5 mg/kg/day and after 2 months of therapy his platelet count is 150 000/mm. Although his course has been long and variable the response to therapy has been designated as good (Fig. 5).

## RESULTS

Table 1 summarizes the results of therapy. The response to therapy is graded according to the platelet count and symptomatology as 1) Excellent, complete hematological and clinical re-

Table 1 Azathioprine (aminon) therapy for refractory idiopathic thrombocytopenic purpura in childhood

Case no	Sex	Age in yrs at onset	Duration of disease	Previous therapy	Platelet count/mm <sup>3</sup>		Azathioprine dose mg/kg/day	Duration of azathioprine therapy (months)	Concomitant therapy	Duration of remission (mo)	Current therapy	Result
					Initial	Current						
1 P B	M	12	24 mo	Prednisone 2 mg/kg	40 000	220 000	2.5 mg	12	None	12	None	Excellent
2 C C	F	10½	23 mo	Prednisone 2 mg/kg	40 000	220 000	2.0-3.0 mg	12	None	3	None	Excellent
3 S D	F	4½	17 mo	Prednisone 2 mg/kg	50 000	250 000	2.0-5.0 mg	8	Splenectomy	12	None	Failure
4 E V	F	2½	5 yrs	Prednisone 2 mg/kg	10 000	210 000	2.5 mg	24	Prednisolone 0.2 mg/kg	3	None	Excellent
5 R A	M	2½	1-½ yrs	Splenectomy Prednisone 2 mg/kg	<10 000	200 000	2.5 mg	27	Prednisolone 0.2 mg/kg	4	Azathioprine 2.5 mg/kg/day	Good

vantageous and resulting in a reduction in the risk of infection since it may not be necessary to suppress all immune responses to obtain a therapeutic response. Immunoglobulins determined at the end of therapy in 3 of our patients were considered within normal levels for IgG with a slightly low level of IgM in one case. Infections were not increased in the total group nor in the patient with low IgM during the 30 months of follow. Therefore the dosage of drug used does not seem to cause irreversible damage to the bone marrow or liver or cause any harmful depression of immunologic responses in the child.

A recent review of the use of azathioprine with renal transplants by Penn *et al* (9) reports the development of a malignant tumor in 5 of the total 120 patients transplanted. Anti-lymphocytic globulin had been given in 4 of these patients. Since oncogenesis is known to occur with a depression of immunologic reactivity by thymectomy, anti-lymphocytic globulin, azathioprine and chronic antigenic stimulation, the role of azathioprine in the development of neoplasia in the described cases is unclear. The use of azathioprine in nephrosis and other autoimmune diseases (1, 4, 6, 7, 8, 11, 13, 15) without the development of neoplasia suggests that a factor other than azathioprine was operative in the development of malignancy in the renal transplant patients.

# SUMMARY

Five children with refractory ITP were treated with azathioprine for 8-27 months. An excellent response was obtained in 3 of the 5 patients, a good response in a fourth. In two pre-splenectomy cases the result was obtained using azathioprine alone and two post-splenectomy cases required low dose steroids in addition to azathioprine. One pre-splenectomy patient failed to respond to the drug alone or in combination with steroids but responded well to splenectomy. A review of the cases in the literature and the experience presented here suggests that azathioprine has a definite role in the therapy of refractory idiopathic thrombocytopenic purpura in childhood.

# REFERENCES

1. Anesbury A C. Current concepts: drugs employed for suppression of immunologic response. *New Eng J Med* 272: 1114, 1965.
2. Baldwin M. Idiopathic thrombocytopenic purpura. *New Eng J Med* 274: 1360, 1966.
3. Bouroncle M A & Doan C A. Refractory idiopathic thrombocytopenic purpura treated with azathioprine. *New Eng J Med* 273: 630, 1966.
4. —. Treatment of idiopathic thrombocytopenic purpura. *JAMA* 207: 2049, 1969.
5. Cohen H & Gardner F H. Thrombocytopenic effect of sustained high dosage prednisone therapy in thrombocytopenic purpura. *Ann Int Med* 263: 611, 1965.
6. Corley C C, Lerner H E & Larson W E. Azathioprine therapy of autoimmune diseases. *Am J Med* 41: 404, 1966.
7. Kuznetsov J A & Ladas S M. Treatment of chronic idiopathic thrombocytopenic purpura with azathioprine and prednisolone: a clinical trial with three children. *Clin Ped* 7: 216, 1968.
8. Mason M, Corry H L, F. Barnes C G., Duncan J F, Hazleman B L & Strickland J D. Azathioprine in rheumatoid arthritis. *Br Med J* 1: 470, 1969.
9. Penn I, Hammond W, Bretschneider L & Harri T E. Malignant lymphomas in transplant patients. *Transplantation Proceedings* 1: 106, 1969.
10. Rendler R W., Luzzo J., Itoga T, Hobson J B & Garrison F E. J. Clinical and hematological study of 6 (1-methyl-4-nitro-5-oxidazoly) thiopurine (B W 31322) and related compounds. *Cancer Chemotherapy Reports* 14: 99, 1961.
11. Schwartz R & Drenthek W. Treatment of autoimmune hemolytic anemia with 6-mercaptopurine and thioguanine. *Blood* 19: 483, 1962.
12. Smith C H, Eriandson M, Schulman I & Stern G. Hazard of severe infection in splenectomized infants and children. *Am J Med* 27: 390, 1957.
13. Swenson L N. Azathioprine in refractory idiopathic thrombocytopenic purpura. *JAMA* 207: 239, 1967.
14. Swenson M A & Schwartz R S. Immunosuppressive therapy: the relation between clinical response and immunologic competence. *New Eng J Med* 277: 163, 1967.
15. Theodor E, Olson E & Waks U. Treatment of ulcerative colitis with azathioprine. *Br Med J* 1: 741, 1969.

Submitted Oct 9, 1969

Accepted Oct 24, 1969

(M W H) Dept of Pediatrics  
Division of Pediatric Hematology  
New York Hospital-Cornell Medical Center  
525 East 68th Street  
New York  
New York 10021  
U S A

Key words: Thrombocytopenic purpura, children, azathioprine.



Table 3 Review of patients treated with azathioprine for chronic idiopathic purpura

Result	No of patients	Authors	Ages
Excellent	15	Bouroncle & Doan	9 23 33 52 70 (4)
		Corley <i>et al</i>	64 (6)
		Kuzenko & Keidan	1½ 6 (7)
		Sussman	15 28 43 (13)
		Present report	2½ 10½ 12
Good	11	Bouroncle & Doan	23 42 50 53 56 59 70 (4)
		Corley <i>et al</i>	64 (6)
		Sussman	35 77 (13)
		Present report	2½
Fair	4	Bouroncle & Doan	42 55 (4)
		Sussman	53 54 (13)
Failure	7	Bouroncle & Doan	7 43 48 (4)
		Corley <i>et al</i>	39 (6)
		Kuzenko & Keidan	10 months (7)
		Sussman	44 (13)
		Present report	4½
Total	37		

shown to have a steroid sparing effect in certain diseases such as ulcerative colitis and rheumatoid arthritis. Theodor *et al* (15) were able to achieve remission with the use of azathioprine in 4 of 7 patients with ulcerative colitis not controlled by steroids alone and demonstrated that steroids could be reduced in these patients. Mason *et al* (8) showed a 35% reduction of steroid dosage in patients with rheumatoid arthritis given azathioprine without adverse side effects. The dose of steroids used temporarily in 2 of our patients was low and is consistent with the findings of other investigators that azathioprine has a sparing effect on steroid dosage. The toxic effects seen in the patients described in this paper have been transitory leukopenia, minimal reduction in hemoglobin and alterations in liver transaminase levels. All corrected when the drug was withdrawn.

Review of the literature (Table 3) revealed 32 cases (27 adults and 5 children) with chronic recurrent ITP who have been treated with azathioprine (4, 6, 7, 13). Of these 32 patients 12 had an excellent response, 10 had a good response, 4 a fair response and 6 had a poor response or failed to respond. The present investigation of 5 children showed that 3 had an excellent result, 1 had a good response

whereas 1 unsplenectomized patient failed to respond but responded excellently to splenectomy. Including the present investigation and patients of all ages 45% had an excellent response, 21% a good response, 17% fair and 18% failed to respond to the use of azathioprine. Response does not seem to be related to duration of disease or age of the patients. Bouroncle & Doan (4) and Sussman (13) have indicated that prolonged therapy may have to be given for an adequate response. Our patients were treated for at least 3 months before the combination of azathioprine plus low dosage steroids was considered a failure and therapy was continued for as long as 27 months for complete remission in a previously splenectomized patient.

The relationship between clinical response and immunologic competence is important in the use of azathioprine. Swinson & Schwartz (14) found inhibition of primary and secondary immune responses with delayed hypersensitivity during therapy with either azathioprine or amethopterin, a decrease in IgG levels and normal synthesis of IgM. The levels of IgG however appeared to be adequate in their patients for antiviral and antibacterial activities. This selective immunosuppressive effect has been considered by these authors to be ad-



Fig. 1 Interscapular telangiectases in a healthy girl aged 9 years.

cance in the closure of the medullary tube, and he found these vascular telangiectases only at sites where the medullary tube had closed. These ectasias were thus instrumental in the covering of nerve tissue by skin and do not represent any error of development.

It must be mentioned, however, that Schiný (21, 22) by means of equally thorough histological investigations of the skin corresponding to the naevi could not demonstrate ectasia of the vessels in infants but demonstrated moderate ectasia of the subpapillary vessels in older children.

Van der Werf (29) mentioned frontal, occipital and sacral types of naevi and was in agreement with Bosard and also influenced by Hochstetter's work in considering that the occurrence of the naevus was connected with the development of the medullary tube.

Theories concerning the mode of development have occasionally been quite unrealistic

for which reason Dixey (9) closed a discussion in the *Lancet* with the words "any midwife will tell that it is caused by an affectionate peck from the stork who delivers the child before taking final leave of its charge. To this Bean (1) remarked that "this seems as good an idea as any."

Several authors (23, 29, 31) considered that hereditary factors must be of significance for the development of nuchal naevus but this presumption is contested by Sklarz (24) who considered that the naevus is so extremely frequent that the question of heredity seems to be of no significance. This is scarcely entirely justified.

#### *Interscapular telangiectases (Fig. 1)*

It was impossible to find interscapular telangiectases described in the current textbooks and handbooks. They are thus not mentioned in Rook's textbook of dermatology from 1968.

REVIEW ARTICLE

NUCHAL NAEVI AND INTERSCAPULAR TELANGIECTASES

*Incidence in Danish School Children*

JAKOB ØSTER and ARNE NIELSEN

*From the Department of Pediatrics Centralsygehuset Randers and the Institute of Human Genetics University of Copenhagen Copenhagen Denmark*

During an investigation of school children concerning haemangiomas attention was drawn to two phenomena viz the frequent incidence of pale red salmon-coloured naevi in the fovea nuchae level with the skin and most frequently localised above the hair margin and the frequent incidence of fine distended blood vessels or telangiectases in the superior interscapular region. Both of these phenomena were surprising because the nuchal naevi so frequently observed in newly born infants, are said to disappear spontaneously according to current text books and general paediatric opinion and because the interscapular region is not a generally recognised site for telangiectases. Paediatric literature on these cutaneous phenomena is extremely scanty. After some search relevant literature mainly from dermatological sources was found.

*Nuchal naevi*

In the literature nuchal naevi are mentioned under many names erythema nuchae capillary telangiectases telangiectatic naevus nuchae, haemangioma nuchae salmon patches of the nape naevus flammeus vinosus nuchae or merely the nape naevus which is termed Unna's naevus in many centres.

As early as 1881 French physicians (1) appear to have described this naevus under the

term *tache sanguine* but the German dermatologist Unna (27) in 1894 presented the first detailed description. He mentioned in his book that Virchow had known this naevus and presented the conception that it developed in sites corresponding to the embryological clefts a theory which has never been generally accepted. Unna considered that these nuchal naevi together with the corresponding naevi in the forehead region, were due to intrauterine pressure phenomena. This theory has not found acceptance latterly one of the reasons being that several authors (3-4) observed naevi in infants delivered by Caesarean section.

As regards the origin Bossard (4) considered dass die Telangiectasen nichts anderes darstellen als mangelhaft zurückgebildete Bezirke aus dem erweiterten Gefässflecht der allmählich vorrückende Vaskularisationszone. In this opinion he was inspired by the investigations undertaken by the Viennese pathologist Hochstetter (11) concerning the vascularization of the cranial skin in human foetuses. Later Sprafke (26) reached a similar conclusion from histological investigations of the skin of the nuchal region in foetuses children and adults. He found that vascular telangiectases were particularly pronounced in the foetuses and interpreted nuchal naevi as resulting from persistence of vascular formations which are of signifi-



Fig. 1 Interscapular telangiectases in a healthy girl aged 9 years

cence in the closure of the medullary tube and he found these vascular telangiectases only at sites where the medullary tube had closed. These ectasias were thus instrumental in the covering of nerve tissue by skin and do not represent any error of development.

It must be mentioned however that Schnyder (21, 22) by means of equally thorough histological investigations of the skin corresponding to the naevi could not demonstrate ectasias of the vessels in infants but demonstrated moderate ectasias of the subpapillary vessels in older children.

Van der Werf (29) mentioned frontal, occipital and sacral types of naevi and was in agreement with Bossard and also influenced by Hochstetter's work in considering that the occurrence of the naevus was connected with the development of the medullary tube.

Theories concerning the mode of development have occasionally been quite unrealistic

for which reason Duxey (9) closed a discussion in the *Lancet* with the words "any midwife will tell that it is caused by an affectionate peck from the stork who delivers the child before taking final leave of its charge. To this Bean (1) remarked that "this seems as good an idea as any".

Several authors (23, 29, 31) considered that hereditary factors must be of significance for the development of nuchal naevus but this presumption is contested by Sklarz (24) who considered that the naevus is so extremely frequent that the question of heredity seems to be of no significance. This is scarcely entirely justified.

#### *Interscapular telangiectases (Fig. 1)*

It was impossible to find interscapular telangiectases described in the current textbooks and handbooks. They are thus not mentioned in Rook's textbook of dermatology from 1968.

Table 1 Incidence of nuchal naevi and interscapular telangiectases in 2 171 Danish school children subdivided according to age and sex

Telangiectases naevi	Boys				Total	Girls				Total
	+	+	-	-		+	+	-	-	
	+	-	+	-		+	-	+	-	
Age										
6	2	2	2	1	7	1	1	—	2	4
7	10	12	11	25	58	9	24	10	28	71
8	8	21	14	26	69	18	16	17	22	73
9	11	21	12	32	76	18	13	13	23	67
10	6	18	34	31	89	18	22	22	26	89
11	9	19	13	34	75	18	20	18	16	72
12	16	24	25	40	105	26	18	24	20	88
13	14	11	29	49	103	32	12	31	19	94
14	33	32	27	83	177	34	46	57	55	192
15	23	36	45	105	209	18	36	70	83	207
16	8	12	28	54	102	10	18	30	49	107
17	1	2	1	13	17	4	—	3	14	21
Total	141	210	241	495	1 087	206	226	295	357	1 084

(19) On the other hand Bern (1) mentioned in his unique work on vascular spiders and related lesions of the skin a phenomenon which he called the cervical fringe and which consists of readily visible veins in hair like whips or linear strands at the base of the neck posteriorly. They may extend down between the shoulderblades or occur only in these regions. His attention had been drawn to the cervical fringe by Holt (12) who gave a good description in the *Lancet* in 1916 of what he called cervical capillary markings in the neighbourhood of the upper dorsal vertebrae.

This communication resulted in a series of observations (6-16) from other authors who supported Holt in his interpretation of the had noticed the same phenomenon and who supported Holt in his interpretation of the phenomenon as a tuberculous manifestation. Riviere (18) considered however that these telangiectases in some cases are very probably of naevoid nature and allied to the venules so often present in the red parts of the cheek and Hughes (14) did not believe that the dilated blood vessels had any connection with tuberculosis but that they might possibly be caused by the pressure and friction of clothing over prominent or relatively unyielding parts, and that constitutional peculiarities of the skin also

probably play a part. Finally the dermatologist Parkes Weber (28) concluded the discussion by drawing attention to the fact that he had described the phenomenon in 1904 and that, in his opinion it was not connected with tuberculosis. His description appears to have been the first but Holt's description which was made independently of Parkes Weber is the best.

## MATERIAL AND METHOD

During the school session 1967-68 one of the authors (J. Ø.) undertook a systematic investigation concerning nuchal naevi and interscapular telangiectases in 2 171 school children aged 6-17 years attending three schools in the town of Rinders. Boys and girls were approximately equally represented. The material is not selected and includes children from both the academic and ordinary classes. All of the children were investigated for the above mentioned conditions and in addition an ordinary clinical investigation was undertaken. The great majority of children were examined in daylight and it must be emphasized that none of the above mentioned type which in the great majority of cases are localized to the skin above the hair margin may be particularly difficult to detect particularly in boys with short thick hair. The telangiectases are most easily seen when the child is requested to press the shoulders forward so that the skin in this region is stretched. There is thus a possibility of clinical errors in diagnosis which make the relevance of clinical assessment debatable. Minimum figures are probably relevant.

Table 2 Incidence of nuchal naevi and interscapular telangiectases in Danish school children

	Girls	Boys	Total
Nuchal naevi	501	382	883
Interscapular telangiectases			
Slight	317	269	586
Moderate	103	72	175
Pronounced	12	10	22
Total	432	351	783
out of	1 084	1 087	2 171
Percentage with naevi	46.2	35.1	40.7
Percentage with telangiectases	39.9	32.3	36.1

## RESULTS

The entire material is recorded in Table 1 and comparison of the incidence of nuchal naevi and telangiectases in the interscapular region is given in Table 2.

In order to investigate whether there is any connection between the two conditions the incidence of naevi expressed as a percentage of the individuals with and without telangiectases was calculated for each age group and sex. The results are apparent from Fig. 2 *a* for boys and Fig. 2 *b* for girls where the abscissa gives the age and the ordinate the incidence of the naevi. In addition two horizontal lines indicate the incidence of naevi for all age groups to-

gether and for each of the two groups of individuals with and without telangiectases respectively.

Where boys are concerned the incidence of naevi is greater in nine out of the 12 age groups among those who have telangiectases than among those without and lower in the remaining three groups.

Where girls are concerned the incidence of naevi is greater in seven out of the 12 age groups among those with telangiectases than among those without and lower in the remaining five groups.

Significant differences between the incidences were found in the following sex and age groups. Boys aged 10 years: significance at 5 per cent level; lower incidence of naevi among those with telangiectases than without; boys aged 14 years: significance at 0.1 per cent level; higher incidence of naevi among those with telangiectases than those without; girls aged 17 years: significance at 5 per cent level; higher incidence of naevi among those with telangiectases than among those without. Taken as a whole the material provides no support for the presumption that there is any connection between the incidence of naevi and telangiectases.

In Fig. 3 *a* the incidence of naevi is expressed as a percentage as compared with the age the sexes being considered separately and as

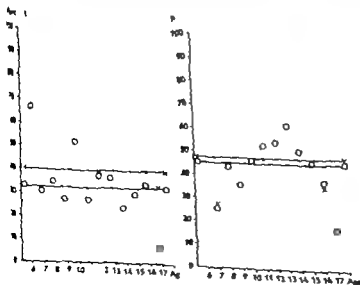


Fig. 2 (a) Boys (b) Girls. Percentage with naevi according to age among schoolchildren with telangiectases.  $\circ$  schoolchildren without telangiectases. Horizontal line indicates overall percentage for all ages.

Table 1 Incidence of nuchal naevi and interscapular telangiectases in 2 171 Danish school children subdivided according to age and sex

Telangiectases naevi	Boys				Total	Girls				Total
	+	+	-	-		+	+	-	-	
	+	-	+	-		+	-	+	-	
Age										
6	2	2	2	1	7	1	1	—	2	4
7	10	12	11	25	58	9	24	—	2	4
8	8	21	14	26	69	18	16	10	28	71
9	11	21	12	32	76	18	13	17	22	73
10	6	18	34	31	89	18	22	22	26	83
11	9	19	13	34	75	18	20	18	16	72
12	16	24	25	40	105	26	18	24	20	88
13	14	11	29	49	103	32	12	31	19	94
14	33	32	27	85	177	34	46	57	55	192
15	23	36	45	105	209	18	36	70	83	207
16	8	12	28	54	102	10	18	30	49	107
17	1	2	1	11	17	4	—	3	14	21
Total	141	210	241	495	1 087	206	226	295	357	1 084

(19). On the other hand Bean (1) mentioned in his unique work on vascular spiders and related lesions of the skin a phenomenon which he called the cervical fringe and which consists of readily visible veins in hair like whorls or linear strands at the base of the neck posteriorly. They may extend down between the shoulderblades or occur only in these regions. His attention had been drawn to the cervical fringe by Holt (12) who gave a good description in the *Lancet* in 1916 of what he called cervical capillary markings in the neighbourhood of the upper dorsal vertebrae.

This communication resulted in a series of observations (6-16) from other authors who supported Holt in his interpretation of the had noticed the same phenomenon and who supported Holt in his interpretation of the phenomenon as a tuberculous manifestation. Riviere (18) considered, however that these telangiectases in some cases are very probably of naevoid nature and allied to the venules so often present in the red parts of the cheek and Hughes (14) did not believe that the dilated blood vessels had any connection with tuberculosis but that they might possibly be caused by the pressure and friction of clothing over prominent or relatively unyielding parts and that constitutional peculiarities of the skin also

probably play a part. Finally the dermatologist Parkes Weber (28) concluded the discussion by drawing attention to the fact that he had described the phenomenon in 1904 and that in his opinion, it was not connected with tuberculosis. His description appears to have been the first but Holt's description which was made independently of Parkes Weber is the best.

## MATERIAL AND METHOD

During the school session 1967-68 one of the authors (J. Ø.) undertook a systematic investigation concerning nuchal naevi and interscapular telangiectases in 2 171 school children aged 6-17 years attending three schools in the town of Randers. Boys and girls were approximately equally represented. The material is not selected and includes children from both the academic and ordinary classes. All of the children were investigated for the above mentioned conditions and in addition an ordinary clinical investigation was undertaken. The great majority of children were examined in daylight and it must be emphasized that naevi of the above mentioned type which in the great majority of cases are localised to the skin above the hair margin may be particularly difficult to detect particularly in boys with short thick hair. The telangiectases are most easily seen when the child is requested to press the shoulders forward so that the skin in this region is stretched. There is thus a possibility of clinical errors in diagnosis which make the relevance of clinical assessment debatable. Minimum figures are probably relevant.

Table 3 *Investigations of incidence of nuchal naevi*

The incidences are given as percentages and the figures in brackets are absolute figures

	Newly born infants			School children			Adults		
	Boys	Girls	Total	Boys	Girls	Total	Males	Females	Total
Just 1994 (77)									10.20
Just 1910 (20)									57 (400)
Lowndes 1918 (4)	46.5	54	50 (130)	17	40.5	27 (504)	15.5	24.5	20 (431)
Reichmann 1978 (10)				4.3	21.4	22.8 (246)	24.1	26.8	25.8 (400)
Holtzner 1930 (13)									5.7 (175)
Conner 1934 (8)			2.8 (210)						
Sprafkin 1937 (76)			62 (152)	50	50	50	38	33	50 (60)
Wesley 1940 (3)									
van der Werf 1952 (29)				40 (78)	41 (237)	41 (521)		46 (152)	
Post, 1953 (17)			42 (879)						
Zachleder 1957 (31)									50 (600)
Smith & Mansfield 1961 (25)			40 (242)						20
Osier & Nielsen 1970				35.1 (1 087)	46.2 (1 084)	40.7 (2 171)			

same in the two groups in each separate age group. It must therefore be presumed that the incidence of telangiectases in these age groups is greater in girls than in boys.

## DISCUSSION

### *Nuchal naevi*

A comparison of the investigations of incidence which includes the present one is presented in Table 3. As it will appear from Table 3, the incidences found vary greatly not only within the same category but also in different categories, e.g. newly born infants, school children and adults respectively. The first difference may be

due to the fact that the number of individuals investigated has varied greatly and in several instances the absolute figures are not mentioned. Another reason may be that investigation in certain children is rendered difficult by prolific growth of hair and finally the extent and form of the naevi may vary from small discrete patches to large confluent areas. In the present work all cases were included irrespective of size or form. This may be why the incidence is reported so differently in different age groups. Bonnard's work (4) which is the most thorough in this respect suggests that the incidence decreases throughout the various age groups from newly born infants to adults.

As regards school children there is good agreement between the incidences found in the present investigation and those found by van der Werf (29) which were also based upon an extensive material. We are unable to explain the deviations attributable to age found in girls in the present investigation with an increase in incidence at the ages of 12-13 years.

Table 4 *Incidence of skin conditions of the scalp and nuchal region in children with nuchal naevi*

	Girls	Boys	Total
Naevus localized scaling	12	3	15
Naevus generalized			
psoriasis of scalp	4	1	5
Naevus nuchal			
dermatitis		0	2
Naevus psoriasis of scalp	0	1	1
Total	16	5	25



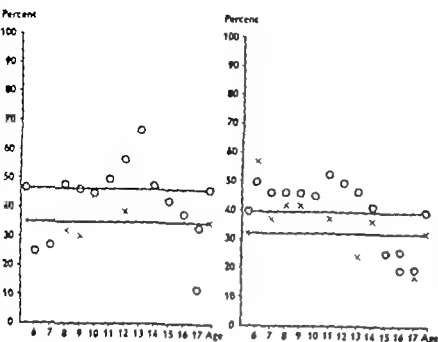


Fig. 3 (a) Percentage with naevi among schoolchildren according to age. (b) Percentage with telangiectases among schoolchildren according to age.  $\times$  boys,  $\circ$  girls.  $\odot$  and  $\circ$  significant deviation from overall percentage. Horizontal line indicates overall percentage for all ages.

ilarly in Fig. 3b the incidence of telangiectases are expressed as percentage as compared with age, the sexes being considered separately. In addition in both Figs. the incidence for all age groups is indicated by two horizontal lines, one for each sex.

Calculations of significance were undertaken for whether the variations for a given sex were greater in the various age groups than might be anticipated from the binomial distribution if the incidence in the population was the same in all age groups. (In the test the incidences  $h$  were converted to the magnitude  $2 \arcsin \sqrt{h}$  whereupon the distribution of this magnitude is approximated with the normal distribution.) As regards the incidence of naevi in boys no significance was found at the 5 per cent level was found while for girls significance for the 1 per cent level was found. As regards the incidence of telangiectases significance was found at the 0.1 per cent level was found for both girls and boys. The particularly deviant values are indicated by extra circles on the legends in Figs. 3a and 3b in order to elucidate possible systematic examination.

For boys with naevi the value at the age of 17 years is rather low and the variation around the average value gives the impression of being slightly greater than would be anticipated from binomial distribution although this was not

significantly greater. The values do not provide evidence against the presumption that the incidence is independent of age for boys in the age groups concerned.

For girls with naevi there is significant deviation downwards at the age of 7 years, upwards at the ages of 12 and 13 years and downwards at the age of 16 years. This suggests a variation due to age with an increase at the ages of 12–13 years and a subsequent decrease to more or less the same level. The values at the age of 6 years (4 children) and 7 years (71 children) are however low compared with the subsequent values.

For boys and girls with telangiectases the course is more or less parallel. The presumption of constant values up to the age of 14 years with a subsequent decrease where both sexes were concerned appears to be reasonable taking into consideration the particularly deviant values in calculation of significance. Apart from the values at the age of 6 years (only 7 boys and 4 girls) the incidence in girls of all age groups in the horizontal plane (6–14 years) was greater than the incidence in boys. The probability that the sign for the difference in incidence in girls minus incidence in boys in one or none of the nine age groups should be the opposite of that in the remaining groups is only 3.9 per cent provided that the incidence is the

Table 3 Investigations of incidence of nuchal naevi

The incidences are given as percentages and the figures in brackets are absolute figures

	Newly born infants			School children			Adults		
	Boys	Girls	Total	Boys	Girls	Total	Males	Females	Total
Ureca 1844 (27)									10
Eschfeld 1910 (20)									57
									(400)
Bossard 1918 (4)	46.5	54	50 (330)	12	40.5	27 (304)	15.5	24.5	20 (431)
Hartmann 1978 (10)				4.3	21.4	8 22.8 (246)	3	26.8	25.8 (400)
Holtzner 1930 (13)									5.7 (175)
Cooper 1934 (8)			2.8 (210)	50	50	30	38	55	50 (60)
Sprafkin 1937 (26)			62 (154)						
Bert y 1940 (3)				40 (288)	44 (233)	42 (571)		46 (152)	
van der Werf 1957 (79)			4 (879)						
Pratt 1953 (17)									50 (600)
Zimke 1957 (31)									20
Schach & Macfield 1962 (25)			40 (247)						
Oster & Nielsen 1970				35.1 (1087)	46.2 (1084)	40.7 (2171)			

same in the two groups in each separate age group. It must therefore be presumed that the incidence of telangiectases in these age groups is greater in girls than in boys.

## DISCUSSION

### Nuchal naevi

A comparison of the investigations of incidence which includes the present one is presented in Table 3. As it will appear from Table 3 the incidences found vary greatly not only within the same category but also in different categories e.g. newly born infants, school children and adults respectively. The first difference may be

due to the fact that the number of individuals investigated has varied greatly and in several instances the absolute figures are not mentioned. Another reason may be that investigation in certain children is rendered difficult by prolific growth of hair and finally the extent and form of the naevi may vary from small discrete patches to large confluent areas. In the present work all cases were included irrespective of size or form. This may be why the incidence is reported so differently in different age groups. Bossard's work (4) which is the most thorough in this respect suggests that the incidence decreases throughout the various age groups from newly born infants to adults.

As regards school children there is good agreement between the incidences found in the present investigation and those found by van der Werf (29) which were also based upon an extensive material. We are unable to explain the deviations attributable to age found in girls in the present investigation with an increase in incidence at the ages of 12-13 years.

Table 4 Incidence of skin conditions of the scalp and nuchal region in children with nuchal naevi

	Girls	Boys	Total
Naevus localis of nuchal	12	3	15
Naevus generalized			
pruritus of scalp	4	1	5
Naevus nuchal			
periorificial	2	0	2
Naevus + pruritus of scalp	11	1	12
Total	18	5	23

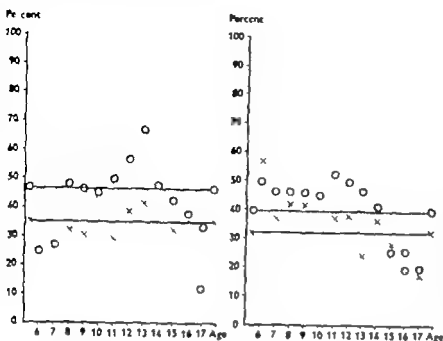


Fig. 3 (a) Percentage with naevi among schoolchildren according to age. (b) Percentage with telangiectases among schoolchildren according to age. Boys  $\circ$ , girls  $\times$  and  $\odot$  and  $\otimes$  significant deviation from "overall" percentage. Horizontal line indicates overall per centage for all ages.

milarly in Fig. 3 *b* the incidence of telangiectases are expressed as percentage as compared with age the sexes being considered separately. In addition in both Figs. the incidence for all age groups is indicated by two horizontal lines one for each sex.

Calculations of significance were undertaken for whether the variations for a given sex were greater in the various age groups than might be anticipated from the binomial distribution if the incidence in the population was the same in all age groups. (In the test the incidences  $h$  were converted to the magnitude  $2 \arcsin h$  whereupon the distribution of this magnitude is approximated with the normal distribution.) As regards the incidence of naevi in boys no significance as regards the 5 per cent level was found while for girls significance for the 1 per cent level was found. As regards the incidence of telangiectases significance as regards the 0.1 per cent level was found for both girls and boys. The particularly deviant values are indicated by extra circles on the legends in Figs. 3 *a* and 3 *b* in order to elucidate possible systematic examination.

For boys with naevi the value at the age of 17 years is rather low and the variation around the average value gives the impression of being slightly greater than would be anticipated from binomial distribution although this was not

significantly greater. The values do not provide evidence against the presumption that the incidence is independent of age for boys in the age groups concerned.

For girls with naevi there is significant deviation downwards at the age of 7 years upwards at the ages of 12 and 13 years and downwards at the age of 16 years. This suggests a variation due to age with an increase at the ages of 12-13 years and a subsequent decrease to more or less the same level. The values at the age of 6 years (4 children) and 7 years (71 children) are however low compared with the subsequent values.

For boys and girls with telangiectases the course is more or less parallel. The presumption of constant values up to the age of 14 years with a subsequent decrease where both sexes were concerned appears to be reasonable taking into consideration the particularly deviant values in calculation of significance. Apart from the values at the age of 6 years (only 7 boys and 4 girls) the incidence in girls of all age groups in the horizontal plane (6-14 years) was greater than the incidence in boys. The probability that the sign for the difference in incidence in girls minus incidence in boys in one or none of the nine age groups should be the opposite of that in the remaining groups is only 3.9 per cent, provided that the incidence is the

ders thorax cheeks and/or ears. Among the 1388 children without telangiectases in the interscapular region only 4 (2 girls and 2 boys) had similar telangiectases elsewhere.

### SUMMARY

Naevi in the lower nuchae and telangiectases in the superior interscapular region have been found with great frequency in 2171 school children aged 6-17 years during a systematic search for these phenomena. The incidence of nuchal naevi was 46.2 per cent in girls, 35.1 per cent in boys. The incidence of telangiectases was 39.9 per cent in girls and 32.3 per cent in boys.

No connection was found between the incidence of naevi and telangiectases.

Concerning nuchal naevi the incidence seems to be independent of age for boys while for girls there seems to be an increase at the ages of 12-13 years and a subsequent decrease.

For boys and girls with interscapular telangiectases the course is parallel with constant values up to the age of 14 years with a subsequent decrease.

A comparison is made of other investigations concerning these phenomena, the significance of which is discussed.

### REFERENCES

1. Bean W B. *Vascular spiders and related lesions of the skin*. Charles C Thomas, Springfield, Ill, 1958.
2. Bessier E & Doyon A. *Leçons sur les maladies de la peau*. Masson, Paris, 1951.
3. Berley F R. Erythema nuchae. *Brit J Derm* 5: 363, 1940.
4. Bonnard Y. Die blauen Feckflecke der Kinder. *Jahrb f Kinder* 88: 204, 1918.
5. Brock W. Zur Frage der Entstehung der Naevi. *Dermat Z* 47: 184, 1916.
6. Chisholm C. The significance of cervical capillary markings. *Lancet* i: 797, 1916.
7. Cleveland D E H. Lichen simplex chronicus. *Arch Derm* 33: 316, 1936.
8. Conner E F. Naevus flammeus atrophic: its occurrence and abnormalities. *Am J Med Sci* 187: 121, 1934.
9. Duvy J R B. The Nape Naevus. *Brit Med J* i: 103, 1955.
10. Huthausen H. Telangiectasen am Nacken. *Der Wochr* 86: 479, 1928.
11. Hochstetter F. *Über die Vascularisation der Haut des Schädeldaches menschlichen Embryonen*. Denkschriften der Kaiserlichen Akademie der Wissenschaften Vol. 93. Alfred Holder, Wien, 1916.
12. Holt R C. The significance of cervical capillary markings. *Lancet* i: 534, 1916.
13. Holtzmann H. *Nackenflecke*. Georg Thieme, Erlangen, 1933.
14. Hughes E. The significance of cervical capillary markings. *Lancet* i: 797, 1916.
15. Lynch F W. Suboccipital dermatitis. *Arch Derm* 68: 307, 1949.
16. Menchen C N. The significance of cervical capillary markings. *Lancet* i: 589, 1916.
17. Pratt A G. Birthmarks in infants. *Arch Derm* 67: 302, 1953.
18. Giviere C. The significance of cervical capillary markings. *Lancet* i: 697, 1916.
19. Rock A, Wittenborn D S & Ebling F J G. *Textbook of Dermatology*. Blackwell, Oxford & Edinburgh, 1963.
20. Sealefield E. *Über Naevi angeboren in der Hinterhäupten Nackengegend*. *Bied Klin* 6: 143, 1910.
21. Schnyder V W. Zur Klinik und Histologie der Angiome. *Arch Derm* 198: 51, 1954.
22. Schnyder V W. Zur Klinik und Histologie der Angiome. *Arch Derm* 200: 483, 1955.
23. Stalar J & Doug A. The Nape Naevus. *Brit Med J* i: 1221, 1955.
24. Silberz E. Telangiectase ("Nape") Naevi. *Brit Med J* i: 1221, 1955.
25. Smith B A & Manfield P A. The natural history of salmon patches in the first year of life. *Brit J Derm* 74: 31, 1962.
26. Sprafke H. Klinische und histologische Untersuchungen über den Hinterhäuptennackennaevus (Naevus Unna) und seine Beziehungen zur Spina bifida. *Arch Derm* 175: 168, 1937.
27. Unna G P. *Die Histopathologie der Haut und ihren*. Verlag von August Arnold, Berlin, 1894.
28. Weber F. Parkes. The significance of cervical capillary markings. *Lancet* i: 599, 1916.
29. Wert E van der. *En Onderzoek naar Huidafwijkingen bij Schoolkinderen*. Thesis, Amsterdam, 1952.
30. Wiehl R. Die Nackenhaut in ihrer besonderen neuroten Reaktionsfähigkeit. *Z Haut Geschlecht* 18: 61, 1955.
31. Zunkeller R. A propos de la fréquence et de l'hérédité du Naevus Vasculosus Nuchae (Unna). *J Genet Hum* 6: 1, 1957.

Submitted Oct. 30, 1969

Accepted Jan. 14, 1970

U (S) Dept. of Paediatrics  
Centralsygehuset  
Randers  
Denmark

**Key words:** Erythema nuchae, haemangioma nuchae, naevus nuchae, nape naevus, Unna's naevus, telangiectases.

As regards the significance of naevi it may be mentioned that Unna's (27) interest in nuchal naevi was aroused during the treatment of pityriasis of the scalp where a red patch frequently persisted despite intensive treatment. Corson (8) mentioned that the skin over nuchal naevi frequently was affected by dermatitis, seborrheic or eczematoid and Wiehl (30) also mentioned the connection between Unna's naevus and eczema of the nape.

Various authors (7-15) have emphasized that the nuchal region is the site of predilection for neurodermatitis (lichen simplex) and that this condition in this site only occurs in women or usually is almost confined to women (19). Lynch (15) considered that the most suitable name for lichen simplex chronicus of the nape would be suboccipital dermatitis and based this on the fact that next to itching redness was the commonest clinical feature and was usually associated with thickening and frequently scaling. In most instances the plaque was practically limited to the fossa. He considered that the inflammatory reaction at this site is more pronounced than in lichenization elsewhere and has observed histologically that capillary dilatation was commonly present, and was frequently associated with congestion. He did not mention any connection between nuchal naevi and lichen of the nape. Nevertheless it was in fact this connection which he had observed clinically and histologically. In his attempt to explain lichen of the nape and its predominance in women is caused by endocrine disturbances he had in fact touched on this connection in his reference that endocrine agents often manifest selectivity of site of action usually influenced by anatomic structure but sometimes by way of vasomotor phenomena.

It is apparent from Table 4 that in the present material there were 15 children with nuchal naevi, the majority of whom were girls who had localised scaling corresponding to the naevus and 2 cases, both girls, had neurodermatitis in the same region. Further a girl aged 16 years with eczema and nuchal neurodermatitis but

without naevi was found and also a boy aged 10 years with generalized eczema and nuchal naevus but with normal skin over the naevus. In two girls aged 15 years who had no nuchal naevi, naevus flammeus of a darker colour than is seen in nuchal naevus localised to the skin between the scapulae in both over the left scapula in one and over the right hip region in the other. No other diseases of the skin or hair were encountered in the children.

Nuchal naevi thus appear to be of significance in the development of pityriasis localised to the same region while on the other hand, it is more doubtful whether there is a connection between nuchal naevi and neurodermatitis in the age group investigated.

#### *Interscapular telangiectases*

Riviere (18) in a material of 164 healthy school children found these dilated venules in 71 (43.3 per cent). These were "strongly marked" in 16, "slight" in 30 and "merely present" in 25. Later Bean (1) mentioned that the relation to age is more obvious in men for he has observed a number of young women in the late teens and twenties with them though they are commoner in elderly women. In men they are rare before the fifties.

The incidence among the school children investigated here is of the same magnitude as in Riviere's work. But as is apparent from Table 2, in the vast majority of the cases the telangiectases are only slightly pronounced and may easily be overlooked if not particularly looked for. In contrast to Bean's remarks the present authors found a constant incidence up to the age of 14 years, always slightly higher in girls than in boys and thereafter a steady decrease in both sexes.

The genesis and significance are obscure. Tuberculosis and other pulmonary disease may be excluded as having any relationship to interscapular telangiectases.

It may be noted that among the 783 children with telangiectases in the interscapular region there were 10 (6 girls and 4 boys) with pronounced telangiectases elsewhere on the shoulder

Table 1 *Fluoride content of infant food*

Estimated average values. Dry milk formulas S and F denote two of the most popular brands on the Swedish market

	Proportion	F content mg/kg mg/l	F content in relation to breastmilk
Breastmilk		0.025	
Cow's milk		0.03	
Cow's milk + water 0.25 ppm F	1+1	0.14	5.5
Cow's milk + water 1 ppm F	1+1	0.57	21
Cow's milk + water 5 ppm F	1+1	2.52	100
Dry milk formula S		0.40	
Dry milk formula S + water 0.25 ppm F	1+6	0.32	13
Dry milk formula S + water 1 ppm F	1+6	1.07	43
Dry milk formula S + water 5 ppm F	1+6	5.07	200
Dry milk formula F		5.0	
Dry milk formula F + water 0.25 ppm F	1+6	1.05	42
Dry milk formula F + water 1 ppm F	1+6	1.8	72
Dry milk formula F + water 5 ppm F	1+6	5.8	230

Table 2 *Classification of enamel fluorosis*

Individual index is determined by the most pronounced type found in two teeth. Group index is the average of individual index numbers

	Index
Normal enamel	0
Questionable fluorosis (very small white dots)	0.5
Very mild fluorosis (small opaque spots distributed over less than 25% of the surface)	1
Mild fluorosis (larger white spots covering less than 50% of the surface)	2
Moderate fluorosis (the whole surface affected often brown discolouration)	3
Severe fluorosis (the whole surface affected brown discolouration hypoplasia entered)	4

dren as regards the parameters of enamel fluorosis must imply a wide latitude of tolerance to fluoride supply during the first year of life. All previous experience indicates that skeletal fluo-

rosis—the main health hazard of excessive fluoride ingestion—is not found in a population as long as enamel fluorosis does not reach high average index values (5).

It may be questioned whether ordinary drinking waters contain substances other than fluorine which can be supplied in greatly increased quantity with formula feeding. Manganese is theoretically most likely since its concentration in drinking water may be more than 50 times as high as in human milk where it has been reported to be 7 µg/l (1). However no data on the toxicity of manganese seem to cause any concern in this respect (6).

## SUMMARY

Infant feeding with water-diluted cow's milk or still more dry milk formulas supplies many

Table 3 *Distribution of enamel fluorosis in Uppsala children (U±S.E.)*

		Index	Mean no. of affected 1st perm. molars/child	Mean no. of affected perm. upper central incisors/child
All B-children	131	1.02±0.06	2.40±0.13	1.24±0.08
All F-children	129	1.17±0.07	2.64±0.13	1.43±0.07
		t=1.60	$\chi^2=60$ DF=4	$\chi^2=2.01$ DF=2
		p>0.1	p>0.1	p>0.3
B-children	18	1.00±0.20	2.8±0.40	1.17±0.23
F-children	74	1.17±0.10	2.88±0.16	1.42±0.10
		t=0.783	$\chi^2=4.46$ DF=4	$\chi^2=1.512$ DF=2
		p=—	p=—	p=—

## SHORT COMMUNICATION

### INCREASED FLUORIDE INGESTION BY BOTTLE FED INFANTS AND ITS EFFECT

Y ERICSSON and U RIBELIUS

*From the Department of Cariology Karolinska Institutet Stockholm and  
the County Dental Services Uppsala Sweden*

It has recently been found that the fluoride content of breastmilk is well below 0.05 ppm much less than previously accepted and about the same as the ionised fluoride content of plasma and the fluoride content of saliva and hardly appears to be influenced by the mother's fluoride ingestion (4). This low fluoride content of the breastmilk implies a many times smaller fluoride dose than that obtained with the dry milk feeding which has become dominant in later years both in Sweden and in many other countries. Calculated fluoride doses appear in Table 1.

Since these variations of fluoride supply coincide with a period when the enamel of many of the permanent teeth is mineralizing the first symptom to be expected if any should be an increased frequency and degree of enamel fluorosis. The purpose of the present investigation has been to compare the occurrence and degree of enamel fluorosis in schoolchildren whose diet as infants has been dominated by either breastmilk or water diluted dry milk formulas.

#### MATERIAL AND METHODS

The main investigation was carried out on 8-9 year old children in Uppsala. This city has for many years had a natural content of about 1.2 ppm F in the drinking water which means that a formula fed child in Uppsala will ingest at least 50 times more fluoride than a breast fed child.

Two groups of children were selected for the

examinations on the basis of a detailed questionnaire to the parents.

1 children who were breast fed for at least 5 months with no period of exclusive feeding with water diluted dry milk preparations (B children).

2 children who received exclusively water-diluted dry milk formulas for at least 5 months (F children).

Later a sub classification was made in order to compare still more extreme groups. As B+ children were registered those B children for whom no period of even partial feeding with dry milk formulas was reported and as F+ children those F children who received water diluted dry milk formulas exclusively for at least 9 months of their first year of life.

The examination of the children was carried out during the autumn term 1969 by one of the authors (U R) who did not know the group classification of the children. Enamel fluorosis was classified according to Dean (2, 3 see Table 2). Cases which appeared intermediate according to this scheme were classified as 1.5, 2.5 or 3.5.

#### RESULTS

Average values of enamel fluorosis index and numbers of mottled first permanent molars and permanent upper central incisors appear in Table 3. No significant difference existed even between the selected groups B+ and F+ but there is throughout a tendency to more pronounced enamel fluorosis in the F children.

#### DISCUSSION

The absence of any significant differences between typically breastfed and formula fed chil-

## CASE REPORT

### NEONATAL MENINGITIS CAUSED BY SALMONELLA THOMPSON

C. G. BERGSTRAND and KARL OLOF NILSSON

From the Department of Paediatrics, Malmö General Hospital, Malmö, Sweden

The cause of purulent meningitis in the newborn period may be any of the common pathogens of neonates in later life but it is well known that the infection is caused mostly by gram negative bacilli belonging to the colon group of organisms. Of these the *E. coli* is by far the most common but typhoid bacillus and various types of the salmonella group have also been reported as causative agents. Purulent meningitis caused by salmonella organisms is relatively rare (7-10%) but deserves nevertheless a certain attention as its treatment may involve complex therapeutic problems in the newborn period. The purpose of this case report is to emphasize some of these problems.

## CASE REPORT

The patient, a girl, was born Sept. 11 1967 23 days after expected confinement. Pregnancy and delivery were without complications. Birth weight 3 010 g and length 49 cm. During the first 9 days of life nothing abnormal was observed. The girl was nursed by the mother without difficulties and had started to gain weight when she fell ill on the 10th day with high fever. She was admitted to hospital on the same day and showed a slightly impaired general condition and signs of light dehydration. The cerebrospinal fluid was turbid and contained 700 cells/mm<sup>3</sup> mostly polymorphous leucocytes (the protein content was 518 mg/100 ml and sugar 97 mg/100 ml). Direct microscopy revealed gram negative bacilli and on the suspicion that the infection was caused by *E. coli* parenteral treatment with a combination of cephalothin, benzyl penicillin and sulfoxadiazine (700 mg/kg body weight/24 h) was instituted. The following day cephalothin was exchanged for oxytetracycline (40

mg/kg body weight/24 h) and ampicillin (170 mg/kg body weight/24 h) was introduced instead of benzyl penicillin (Fig. 1). Culture of the cerebrospinal fluid yielded an organism belonging to the salmonella group and was identified as *S. Thompson*. Cultures of blood and stool were also positive for *S. Thompson*. The organism was highly sensitive to oxytetracycline, ampicillin and sulfoxadiazine and after treatment for 60 hours the patient's temperature was normal. A new lumbar puncture was performed after 6 days of treatment but culture of the cerebrospinal fluid still demonstrated growth of *S. Thompson*. Oxytetracycline was then exchanged for chloramphenicol which was given for 11 days (total amount 2.8 g). The treatment with ampicillin and sulfoxadiazine was continued for 1 month. During this period repeated cultures of the cerebrospinal fluid were reported sterile and the number of leucocytes decreased to 41/mm<sup>3</sup> with only a few neutrophils. The protein and sugar content of the cerebrospinal fluid decreased to 120 mg/100 ml and 41 mg/100 ml respectively. Chlorides were 119 mEq/l. The infant's general condition improved and she gained weight.

Four days after all treatment had been discontinued the patient started to vomit and ran a high fever. Lumbar puncture revealed a turbid cerebrospinal fluid with 3 300 cells/mm<sup>3</sup> mostly neutrophilic leucocytes. Growth of *S. Thompson* was demonstrated but the blood cultures were this time sterile. Treatment with streptomycin sulfate, ampicillin and sulfoxadiazine was immediately instituted. Streptomycin was given for 8 days (total dose 3.04 g) and during this time three blood transfusions were given with a few days interval. It was decided to continue with ampicillin and sulfoxadiazine for 3 months and during this time the cerebrospinal fluid became normal; all further cultures were sterile and the infant thrived and developed quite normally. Hydrocephalus or other signs of neurological abnormalities have not been observed during the follow-up period of 2 years. Repeated electroencephalograms and echocardiograms have not revealed anything abnormal.

The mother was quite healthy during pregnancy and after delivery but stool cultures taken one day



times greater fluoride doses than breast feeding already with low water fluoride content. An investigation of schoolchildren in Uppsala (1.2 ppm F in the drinking water) showed only an insignificant trend towards increased enamel mottling in typically formula fed children compared to breast fed children. No health hazard thus seems to be involved.

### ACKNOWLEDGEMENT

The authors gratefully acknowledge the support of the administrative, medical and dental chief officers of the Uppsala school authorities. Financial support was obtained from Patentmedelfonden for odontologisk profylaxforskning (Swedish Patent Revenue Research Fund).

### REFERENCES

- 1 Brock A. & Wolff L. K. The quantity of Ca, Zn and Mn in cow's milk. *Acta Brev. Neerl. Pharmacol. Pharmacol. Microbiol.* 5: 80, 1935.
- 2 Dean H. T. Classification of mottled enamel diagnosis. *J. Am. Dent. Ass.* 21: 1421, 1934.
- 3 — The investigation of physiological effects by the epidemiological method. in F. R. Moulton (ed.) *Fluorine and dental health*. Am. Ass. Adv. Sci. Washington D.C. 1942.
- 4 Ericsson Y. Fluoride excretion in human saliva and milk. *Caries Res.* 3: 159, 1969.
- 5 Hodge H. C. & Smith F. *Fluorine chemistry*, vol. IV. Academic Press, New York, 1965.
- 6 Underwood J. E. *Trace elements in human and animal nutrition*. Academic Press, New York and London, 1962.

Submitted Jan. 15, 1970

Accepted Jan. 30, 1970

Department of Cariology  
Karolinska Institutet  
Box 3207  
S-103 64 Stockholm 3  
Sweden

**Key words:** Bottle feeding, fluoride, enamel formation.

Table 2. Immunoglobulin levels in mg/100 ml

Age	IgG		IgA		IgM	
	Patient	Normal mean and range	Patient	Normal mean and range	Patient	Normal mean and range
1 week	360	960 585-1123	2	35 18-62	90	33.4 13.2-67.0
3 weeks	360	—	4	—	32	—
4 weeks	390	702 510-846	17	7.4 1.7-12.7	50	31.1 10.7-57.1
2 months	460	—	48	—	81	—
3 months	330	432 278-672	24	12.4 2.7-28.2	48	37.1 14.9-68.1
3½ months	300	—	30	—	48	—
4 months	310	367 231-578	30	18 2.9-37.7	27	36.8 12.6-56.7
6 months	290	470 2.0-645	30	14.2 4.4-35.8	70	43.8 18.6-69.6
1 year	370	705 453-1125	190	15.8 13.8-65.5	51	59.6 33.5-96.8

Normal values taken from Berg and Johansson & Berg (59)

of the microorganism this combination of drugs ought to have been highly effective. In spite of 6 days' treatment with adequate doses the cerebrospinal fluid did not become sterile which prompted the exchange of oxytetracycline for chloramphenicol. Whether this change of therapy had any influence on the further outcome is an open question.

The optimal duration of treatment of salmonella meningitis is not known (13) but a period of 2-3 weeks after symptoms have subsided is recommended (11-14). In the present case the infant was treated for 4 weeks but nevertheless a relapse occurred which indicates that the recommended duration of treatment may at least in some cases be too short. Generally accepted criteria of successful treatment of purulent meningitis e.g. patient afebrile for 5 days, cerebrospinal fluid cell count of 30 cells or less and normal sugar and protein content (12) appear to be of questionable value in a case of the type reported here. Whether a treatment for 3 months as employed in the present case is really necessary seems impossible to decide but it may be concluded that when a non-toxic drug as ampicillin can be used it is wise to continue the treatment for longer than the usually recommended 4 weeks.

Investigations in recent years have given ample evidence that the newborn infant is capable of an immune response to most infectious agents (2). The response to salmonella antigens has been studied in detail (2, 4) and the newborn infant is apparently able to produce agglutinins against the O antigens as well as the H antigens. In the case presented here only antibodies to S. Thompson O and OH antigens could be demonstrated and the titers did not reach very high levels (Table 1). The observed titers and the negative Widal test 2 years after the infection must however be taken as evidence of an immunological response. Whether this response can be regarded as entirely normal is difficult to decide with certainty as controls with the specific antigen are lacking. An evaluation of the results is also complicated by the three blood transfusions in the beginning of the second month of treatment.

The rise in the titer observed at 6 months of age is best explained as an anamnestic reaction caused by colonization of the intestine with coli bacilli after discontinuation of the antibiotic treatment.

The immunoglobulin levels were first measured during the second week of treatment.

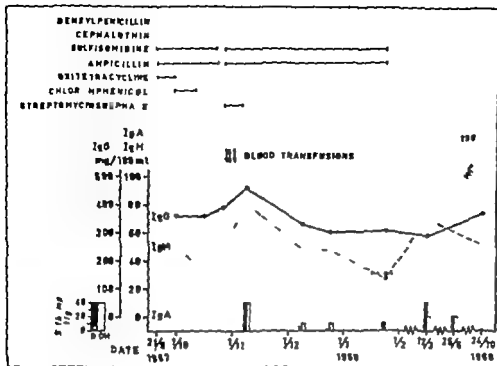


Fig 1

after admittance of the patient showed her to be a carrier of *S. Thompson*. The same pattern of resistance was demonstrated as shown by the organism isolated from her baby. She has agglutinating antibodies to *S. paratyphi B* O antigen and to *S. Thompson* OH antigen corresponding to a previous salmonella infection. Her immunoglobulin levels are within the normal range.

During the course of the disease and for several months afterwards the Widal test and the immunoglobulin levels were followed. The immunoglobulin determinations were performed with Oudin tube technique as described by Wollheim (15). The results are given in Tables 1 and 2 and in Fig. 1. As shown in Table 1 agglutinating antibodies to *S. paratyphi B*

O and H antigens could not be demonstrated. Agglutination with *S. Thompson* O and OH antigens was first tested after 6 weeks of treatment. The antibody titer never reached very high levels and 2 years after the infection the Widal test was completely negative.

## DISCUSSION

The treatment of purulent meningitis in the newborn period involves several problems. In the majority of patients the infection is caused by gram negative organisms and the choice of antibiotic(s) may be difficult. For salmonella meningitis in the newborn infant ampicillin is recommended and chloramphenicol only when the infecting organism is resistant to ampicillin and then in doses not exceeding 25 mg/kg/day (13). In the case reported here the organism was sensitive to several antibiotics and sulfonamide. Ampicillin as a practically nontoxic drug did not cause any discussion. It was however deemed desirable to use more than one antibiotic and oxytetracycline was chosen as less toxic than streptomycin and chloramphenicol. The risk of dental discoloration was considered of minor importance in this connection and the use of sulfisomidine was thought to be fairly safe as the infant was 9 days old and had no jaundice. Judging from the *in vitro* sensitivity

Table 1. Result of the Widal test

Age	Agglutination titer			
	<i>S. paratyphi B</i>		<i>S. Thompson</i>	
	O	H	O	OH
3 weeks	neg	neg	—	—
5 weeks	neg	neg	—	—
6 weeks	neg	neg	—	—
2 months	—	—	1/40	1/40
3 months	neg	neg	1/5	1/10
3½ months	neg	neg	neg	1/10
4½ months	neg	neg	1/10	neg
6 months	neg	neg	1/40	1/20
9 months	neg	neg	1/20	1/20
2 years	neg	neg	neg	neg

\* The O and H antigens were not completely separated but the H antigen is dominant.

- aphtous by a newborn infant with Salmonella  
et. paed. *J Pediatr* 68 780 1966
- 9 Johansson, S. G. O. & Berg, T. Immunoglobulin  
levels in healthy children. *Acta Paediatr Scand*  
56 572 1967
- 10 Kuentz, M. & Ellger, E. Komplikationen und  
unusuelle Verlaufsformen der Salmonellen  
im Kindesalter. *Arch Hyg Bakt* 151 278 1967
- 11 Kreck, U. & Modde, H. Das Meningitis salmonel-  
losa. Mikrobiologie und Klinik unter Berücksichti-  
gung eigener Beobachtungen. *Helv Paediatr Acta*  
21 85 1966
- 12 Mathes, A. W. & Wehrle, P. F. Management of  
bacterial meningitis in children. *Pediatr Clin N*  
*Amer* 15 185 1968
- 13 *Med Lett Drugs Ther* 10 50 1968
- 14 Smith, E. S. Salmonella meningitis in infancy.  
*Am J Dis Child* 88 732 1954
- 15 Wollheim, P. Studies on the immunoglobulins  
in human serum with special reference to  $\gamma$ M.  
*Acta Univ Lundens* section II no. 5 Lund 1968

Dept. of Paediatrics  
Malmö Allmänna Sjukhus  
214 01 Malmö  
Sweden

**Key words** Neonatal meningitis Salmonella Thompson

when the infant had reached an age of three weeks (Table 2) and were then followed up to 2 years. When the results are compared with the normal values given in the literature (1-3, 9) some questions arise. The IgG levels first found in the patient appear to be unusually low and the expected decrease during the first few months of life generally seen in healthy infants could not be observed. It has been shown by Berg (5) that in infants with relatively low IgG-levels at birth the decrease is slower than in infants with high levels. At the age of 6 weeks the difference between these two groups is small and eventually becomes insignificant. The very slow decrease of the patient's IgG levels may therefore be regarded as physiological but it cannot be excluded that the infection also played a certain role. Allansmith *et al* (1) have found that in some newborns no drop in the IgG levels can be observed and take this as evidence of an IgG synthesis occurring already during the first weeks of life.

It has been shown (2) that 20-30 days after immunization with salmonella antigens the newborn at least in many cases develops antibodies of the IgG type. The production of IgM antibodies is however the more rapidly occurring and also quantitatively more important immune response. It has been observed that newborns produce IgM antibodies both to the flagellar and to the somatic salmonella antigens after artificial immunization as well as after natural infections (6-8). In the present patient the IgM levels did not rise appreciably during the first 6 weeks. It might be argued as the level of IgM during the first week of life is not known that the initial value was very low and that the maximal rise had already occurred before the immunoglobulins were first measured at 3 weeks. The immune response seems nevertheless to be rather poor with regard to the massive infection and it is tempting to relate this to the relapse which occurred after 1 month's treatment. Real evidence of a defective antibody production is however lacking and the immunoglobulin levels found at the age of 2

years are fairly normal even if the IgG values must be regarded as low.

The increase in the IgM level at 2 months coincided with an increase in the IgG and IgA levels and can most readily be explained by the three blood transfusions given during the seventh week.

## SUMMARY

A case of neonatal meningitis caused by *Salmonella Thompson* is presented. In spite of adequate antibiotic treatment during 1 month a relapse occurred and treatment had to be continued for 3 months. The final outcome was very favourable and at the age of 2 years the patient appeared quite normal. During the course of the disease the immunoglobulin levels and the Widal test were followed. Evidence of an antibody deficiency syndrome explaining the patient's relapse could not be demonstrated.

## ACKNOWLEDGEMENT

The authors are indebted to Frank Wallbeum MD for the immunoglobulin determinations.

## REFERENCES

1. Allansmith M, McClellan B H, Butterworth M & Maloney J R. The development of immunoglobulin levels in man. *J Pediatr* 72: 271 1969.
2. Alterneier W A & Smith R T. Immunologic aspects of resistance in early life. *Pediatr Clin N Amer* 12: 633 1965.
3. Bauer U & Freund R. Normalwerte von Immunoglobulinen im Kindesalter. *Archiv Kinderheilk* 117: 559 1969.
4. Bellanti J A & Jackson A L. Characterization of the serum immunoglobulins to the somatic antigen of *S. typhosa* in an infant following intra-uterine immunization. *J Pediatr* 71: 783 1967.
5. Berg T. The immunoglobulin development during the first year of life. *Acta Paediatr Scand* 58: 229 1969.
6. Goloff S P & Cochran W D. Antibody response to the somatic antigen of *Salmonella newport* in premature infants. *Pediatrics* 37: 610 1966.
7. Henderson L L. *Salmonella meningitis*. *Am J Dis Child* 75: 351 1948.
8. Hodes H L, Zepp H D, Ambender E, Berger W & Hevzy M. Production of O and H

Table 1 The results of investigations carried out while the patient was receiving fructose in his diet and 8-12 days after the introduction of a fructose free diet

	After admission	On fructose free diet
<b>Blood</b>		
Haemoglobin (g/100 ml)	8.3	8.3
Platelet count (mm <sup>3</sup> )	71 000	over 250 000
Plasma cit. amn (mg/100 ml) (normal 8.5-10.5)	8.9	—
Plasma phosphorus (mg/100 ml) (normal 4.0-5.5)	1.8	4.4
Plasma sodium (mEq/l)	137	136
Plasma potassium (mEq/l)	3.3	4.7
Plasma chloride (mEq/l)	102	103
Plasma TCO (mEq/l)	15	21
<b>Liver function</b>		
Plasma bilirubin (mg/100 ml)	8.4	1.1
Plasma proteins (g/100 ml)	3.9	5.4
Serum transaminases ( $\mu$ M/100 ml serum/hour)		
GOT (normal 10-110)	294	168
GPT (normal 10-110)	270	84
Prothrombin time (control value)	135	120
Partial thromboplastin time (control value)	160	175
Fibrinogen level (control value)	5	—
<b>Urine</b>		
Glucose (mg/100 ml)	0-200	0
Galactose (mg/100 ml)	0-200	0
Fructose (mg/100 ml)	0-200	0

serum GOT level rose from 150  $\mu$ M/100 ml/hour to 310  $\mu$ M/100 ml/hour and the GPT from 64  $\mu$ M/100 ml/hour to 154  $\mu$ M/100 ml/hour during the first 5 hours after the fructose load. Urine passed during the test contained 300 mg fructose/100 ml.

These results were considered diagnostic for HFI (15).

#### Amino acid studies

Plasma tyrosine levels around 8 mg/100 ml (normal 1 mg/100 ml) and methionine levels around 7 mg/100 ml (normal 0.3 mg/100 ml) were found while the patient was receiving fructose in his diet. The remainder of the plasma amino acid pattern was at the upper limit of normal. A heavy generalized ammo-

aciduria was present at this time and there was also excessive excretion of p-hydroxyphenyl acetic and lactic acids in the urine. Negative results were obtained when the urine was tested with Phenistix or 2,4-dinitrophenylhydrazine.

After 11 days treatment with a fructose free diet the plasma tyrosine had fallen to 2 mg/100 ml but the methionine level remained elevated at 7 mg/100 ml. Thirteen days after the introduction of a fructose free diet the urinary amino acid pattern was at the upper limit of normal and phenolic acids could no longer be detected in the urine. However excess p-hydroxyphenyllactic acid was detected in urine passed after the fructose tolerance test.

#### DISCUSSION

The clinical and biochemical features of HFI and tyrosinosis may be very similar. Both conditions may present during the first months of life with vomiting, failure to thrive, jaundice, enlargement of the liver and ascites. Hypophosphataemia, hypoglycaemia, bleeding disorders and abnormal liver function tests may occur in either condition together with proteinuria, aminoaciduria, mellituria (glucose, galactose or fructose) and renal acidosis (1, 3, 5, 8). The findings in the present case illustrate a further similarity between the two conditions. The elevated plasma levels of tyrosine and excess of phenolic acids in the urine which were present while the patient was receiving fructose or his diet suggested a diagnosis of tyrosinosis (3). The correct diagnosis of HFI was only suspected some time after admission when a detailed history was obtained with the assistance of an interpreter.

Elevated plasma tyrosine levels have been found in adult subjects with chronic liver disease (6) and the abnormality of tyrosine metabolism noted in the present case was probably secondary to a transient fructose induced disturbance of liver cell function. A similar disorder of tyrosine metabolism has been described by Lindemann *et al.* (7) in an infant who

## CASE REPORT

### ABNORMAL TYROSINE METABOLISM IN HEREDITARY FRUCTOSE INTOLERANCE

D. H. GRANT, F. W. ALEXANDER and J. W. T. SEAKINS

*From The Hospital for Sick Children and Institute of Child Health, London  
WC1, United Kingdom*

There have been many accounts of hereditary fructose intolerance (HFI) since the condition was recognised by Froesch *et al* in 1957 (2). The clinical features of cases which present in early infancy were reviewed by Levin *et al* (5) who noted the similarity to those seen in tyrosinosis (3). The following report describes an infant with HFI who showed the biochemical features of tyrosinosis while he was receiving fructose in his diet.

#### CASE REPORT

The patient was born after a 32 week pregnancy and weighed 1800 g. He was initially fed on a diet which did not contain fructose and made good progress, weighing 2500 g at the age of 8 weeks. At this time his diet was changed to one containing sucrose and he began to vomit after feeds. When he was 11 weeks old he was given larger quantities of sucrose as he had become constipated and during the next 5 weeks his vomiting became more frequent and his condition deteriorated. He was admitted to hospital in Athens at the age of 16 weeks with jaundice, hepatomegaly and gross ascites which necessitated paracentesis abdominis. Elevated serum transaminase levels, hypoproteinaemia, proteinuria and glycosuria were noted at that time.

When admitted to this hospital at the age of 17 weeks he appeared ill and moderately jaundiced. His liver extended 7 cm below the costal margin, his spleen tip was palpable and he had marked ascites with communicating hydrocoeles. The history of recurrent vomiting after sucrose ingestion was not obtained at the time of admission and the patient continued to receive sucrose (15 g/day) in his diet. There was no significant change in his condition un-

til 3 weeks after admission when a more complete dietary history was obtained and glucose was substituted for sucrose in his feeds. On this fructose free diet he had no further vomiting and within 2 weeks his jaundice had cleared, his liver had become smaller and softer and his ascites had resolved completely. He was thriving when discharged from hospital at the age of 23 weeks.

#### INVESTIGATIONS

##### General

The results of investigations carried out before the introduction of a fructose free diet are given in Table 1. The patient had mild anaemia and thrombocytopenia. Liver function tests indicated hepato-cellular damage and clotting tests were abnormal. Hypophosphataemia and mild acidosis were also found. Chromatography of the urine for sugars showed a variable mellituria. There was no evidence of rickets on X-rays of the wrists and chest.

##### Fructose tolerance test

A fructose load (0.5 g/kg) was given by nasogastric tube 16 days after fructose had been excluded from the patient's diet. The blood glucose fell from a fasting level of 56 mg/100 ml to 10 mg/100 ml 2 hours after the fructose load. The total blood sugar fell from 60 mg/100 ml to 31 mg/100 ml and the plasma phosphate from 6.0 mg/100 ml to 4.0 mg/100 ml during the same period. The

## CASE REPORT

### SILVER S SYNDROME

S VESTERMARK

*From the Department of Paediatrics, Aalborg Hospital, Glostrup, Denmark.*

Congenital hemihypertrophy is a well known phenomenon which in less severe cases can hardly be characterized as pathological (10). In more severe cases the hemihypertrophy will be of clinical interest especially if other malformations occur simultaneously. In 1953 Silver *et al* (13) described two cases of hemihypertrophy with several other peculiar abnormalities. In 1964 Silver (14) collected a total of 29 cases presenting this entity which is now often termed Silver's syndrome. The following features may be observed in this syndrome (14).

- Significant asymmetry
- Shortness of stature
- Small size despite birth at term
- Variation in the pattern of sexual development
  - Elevated urinary gonadotrophins or
  - Early sexual development or
  - Premature estrogenization of urethral or vaginal mucosa or
  - Markedly retarded bone age in relation to sexual development
- Cafe au lait areas of the skin
- Unusually short fifth fingers and/or
- Increased curve of fifth fingers
- Triangular shape of face
- Turned-down corners of the mouth
- Syndactylism or other abnormalities of the toes

In the following two typical cases of Silver's syndrome are described

## CASE REPORTS

### Case 1

This boy is the younger of two sons. No miscarriages. Family history negative. The parents are of normal height. Pregnancy reported normal. The patient was born at term. Birth weight 2,250 g. Length 48 cm. Normal delivery. The right leg was noticed to be longer than the left at the age of 6 weeks.

On admission at the age of 3 months to the paediatric ward the patient's weight was 3,500 g, height 54 cm. There was syndactyly of the third and fourth fingers at the left side. Hyperflexibility of the finger joints. Left foot 0.5 cm shorter than the right (Fig. 1). Left leg was thinner and 1 cm shorter than the right (Fig. 2). There was a pronounced tendency to perspiration.

At the age of 5 months the boy measured 56 cm, i.e. an increase in height since birth of 8 cm as against the normal increase of 15 cm. Weight 3,800 g. Furthermore there was a distinct palmar mongoloid crease on both hands. The fifth finger was shorter than normal and slightly curved. Chromosomes normal. Serum calcium, phosphorus and alkaline phosphatase normal. Fractionated serum lipids normal. Serum protein electrophoresis normal. Intravenous urography normal. X-ray of chest and skull normal. Ossification centres showed retarded development as compared with his age but identical on both sides. Electrocardiogram normal. Electroencephalogram normal. Mental development normal. As to the motor development he appeared slightly retarded. At the age of 2 years the boy's height was 74 cm and his weight 6,680 g; the rate of increase in height is slower than normal and is now distinctly below the 3rd percentile for the normal curve. The same applies to his weight.

### Case 2

This girl is an only child. Her mother has formerly had three miscarriages. Both parents are tall and stout. During the fifth month of pregnancy the mother experienced transitory pains in the abdomen.



showed low levels of hepatic fructose 1-phosphate aldolase *post mortem* and Halvorsen & Gjessing (4) subsequently reported elevated plasma tyrosine levels and excessive urinary excretion of phenolic acids in three further infants with HFI. It appears that such findings may be common in fructose intolerance and it is clearly important to exclude this diagnosis in any infant with biochemical evidence of tyrosinosis.

### SUMMARY

Elevated plasma tyrosine and methionine levels, together with excessive urinary excretion of *p*-hydroxyphenylacetic and lactic acids were found in an infant with hereditary fructose intolerance (HFI). Such findings may be common in HFI and this diagnosis must be considered in any infant with biochemical abnormalities of tyrosine metabolism.

### ACKNOWLEDGEMENT

We thank the Nuffield Foundation for financial help.

### REFERENCES

- 1 Black J A & Simpson J. Fructose intolerance. *Brit Med J* 4: 138 1967.
- 2 Froesch E R, Freder A, Labhart A, Stuber H W & Wolf H P. Die hereditäre Fructose

intoleranz eine bisher nicht bekannte kongenitale Stoffwechselstörung. *Schweiz. Med. Wschr.* 87: 1168 1957.

- 3 Halvorsen S, Pande H, Loken A C & Gjessing L R. Tyrosinosis. A study of six cases. *Arch Dis Child* 41: 238 1966.
- 4 Halvorsen S & Gjessing L. Studies on the amino levels in serum and urine of infants and children with metabolic liver diseases. *Abstr. European Societies for Paediatric Research and Paediatric Gastroenterology*, p. 43 Sept. 1969.
- 5 Levin B, Snodgrass G J A I, Oberholzer V G, Burgess A E & Dobbs R H. Fructosæmia: observations on seven cases. *Amer J Med* 45: 826 1968.
- 6 Levine R J & Conn H O. Tyrosine metabolism in patients with liver disease. *J Clin Invest* 46: 2012 1967.
- 7 Lindemann R, Gjessing L R, Merton B & Halvorsen S. Fructosæmia/Acute tyrosinosis. *Lancet* i: 891 1969.
- 8 Siss-Kortzik A, Ficker S, Pannier L, Koch S W, Fraser D & Jackson S H. Secondary metabolic derangements in patients with tyrosinemia. *Canad Med Ass J* 97: 1079 1967.

Submitted Jan 3 1970

Accepted Jan 10 1970

(D B G)

The Hospital for Sick Children  
Great Ormond Street  
London WC1  
England

**Key words:** Hereditary fructose intolerance; abnormal tyrosine metabolism; urinary *p*-hydroxyphenylacetic acid; urinary *p*-hydroxyphenyllactic acid.

## CASE REPORT

### SILVER S SYNDROME

S VESTERMARK

*From the Department of Paediatrics Amisrygshuset Glostrup Denmark*

Cerebral hemihypertrophy is a well known phenomenon which in less severe cases can hardly be characterized as pathological (10). In more severe cases the hemihypertrophy will be of clinical interest especially if other malformations occur simultaneously. In 1953 Silver *et al* (13) described two cases of hemihypertrophy with several other peculiar abnormalities. In 1964 Silver (14) collected a total of 29 cases presenting this entity which is now often termed Silver's syndrome. The following features may be observed in this syndrome (14).

- Significant asymmetry
- Shortness of stature
- Small size despite birth at term
- Variation in the pattern of sexual development
  - Elevated urinary gonadotrophins or
  - Early sexual development or
  - Premature estrogenization of urethral or vaginal mucosa or
  - Markedly retarded bone age in relation to sexual development
- Café au lait areas of the skin
- Unusually short fifth fingers and/or
- Increased curve of fifth fingers
- Triangular shape of face
- Turned-down corners of the mouth
- Syndactylism or other abnormalities of the toes

In the following two typical cases of Silver's syndrome are described

## CASE REPORTS

### Case 1

This boy is the younger of two sibs. No miscarriages. Family history negative. The parents are of normal height. Pregnancy reported normal. The patient was born at term. Birth weight 2,250 g. Length 48 cm. Normal delivery. The right leg was noticed to be longer than the left at the age of 6 weeks.

On admission at the age of 3 months to the paediatric ward the patient's weight was 3,500 g, height 54 cm. There was syndactyly of the third and fourth fingers at the left side. Hyperflexibility of the finger joints. Left foot 0.5 cm shorter than the right (Fig. 1). Left leg was thinner and 1 cm shorter than the right (Fig. 2). There was a pronounced tendency to perspiration.

At the age of 5 months the boy measured 56 cm, i.e. an increase in height since birth of 8 cm as against the normal increase of 15 cm. Weight 3,800 g. Furthermore there was a distinct palmar mongoloid crease on both hands. The fifth finger was shorter than normal and slightly curved. Chromosomes normal. Serum calcium, phosphorus and alkaline phosphatase normal. Fractionated serum lipoids normal. Serum protein electrophoresis normal. Intravenous urography normal. X ray of chest and skull normal. Ossification centres showed retarded development as compared with his age but identical on both sides. Electrocardiogram normal. Electroencephalogram normal. Mental development normal. As to the motor development he appeared slightly retarded. At the age of 2 years the boy's height was 74 cm and his weight 6,680 g, the rate of increase in height is slower than normal and is now distinctly below the 3rd percentile for the normal curve. The same applies to his weight.

### Case 2

This girl is an only child. Her mother has formerly had three miscarriages. Both parents are tall and stout. During the fifth month of pregnancy the mother experienced transitory pains in the abdomen.



Fig 1 Case 1 Short left foot

Delivery was normal. The patient was born at term but the birth weight was only 1 320 g and the length 40 cm. The placenta was normal. The toes on the left foot were missing (Fig 3). The right leg was distinctly thinner and shorter than the left. At the age of 9 months the weight was 4 380 g and the height 61 cm. Motor development was retarded but mental development normal. The right leg was 1 cm shorter than the left (Fig 4). The right arm was also shorter than the left and the right side of the face slightly flattened. There was increased tendency to perspiration.

On examination at the age of 18 months normal urinary excretion was found of pituitary gonadotrophins, 17 ketosteroids, 17 ketogenic steroids and estrogens. Chromosomes were normal. Urinary excretion of amino acids normal. EEG normal. X ray of skull and chest normal. X ray of the feet showed absence of the second and third phalanges of the fourth and fifth toes on the right. On the left side all phalanges were missing.

At the age of 18 months the ossification centres were found to be retarded corresponding to the age of 3-6 months but identical on both sides. At the age of 15 months distinct estrogenization of the vaginal mucosa was demonstrated.

Since then the patient has been followed up in our out patient clinic and has presented acceleration of the weight increase and almost normal rate of increase in height. Hence at the age of 2 years the height is 75 cm and the weight 7 500 g.

## DISCUSSION

The first four symptoms are sometimes described as the major symptoms, the others as minor symptoms. According to Reiter & Scherz (9) three major symptoms will be sufficient to establish the diagnosis and hence both our patients fulfil the criteria for the diagnosis of Silver's syndrome.

Occasionally the asymmetry is described as hemihypertrophy but as stated by Ferner et al (3) the term asymmetry is to be preferred since it might be difficult to decide whether a genuine hemihypertrophy is present or whether it is a question of a corresponding hemihypotrophy.

The asymmetry can vary greatly from mild and localized cases to severe total asymmetry (14). Mild cases might escape recognition at birth but will become evident during growth. The asymmetry can be unilateral or crossed (15). Since the asymmetry might occasionally be very slight there is nothing to prevent us



Fig 2 Case 1 The left leg is shorter and thinner than the right

from regarding Russel's intrauterine dwarfism (11) as a variant of Silver's syndrome. However it should be pointed out that Scalay (16) believes the asymmetry to be of decisive importance for establishing the diagnosis of Silver's syndrome. The asymmetry in these cases cannot be distinguished from other types of asymmetry. However the well known relationship between hemihypertrophy and Wilms' tumour (5) has not been observed in Silver's syndrome but this might be due to the fact that the number of cases of the syndrome hitherto described is very limited.

In Silver's syndrome the birth weight is always low in spite of normal duration of pregnancy. According to Black (1) the birth weight must be lower than 2050 g before the term low birth weight can be applied. In our Case 1 the birth weight is slightly above this limit but definitely below normal birth weight and as in the case described by Hook & Yarns (7) the increase in weight during the neonatal period was clearly reduced to below the 3rd percentile. Patients with Silver's syndrome very seldom present birth weights as low as that of our patient No. 2 (1320 g) but in contrast with patient No. 1 the weight gain increased in this patient although at the age of 2 years she had not yet attained the 3rd percentile. In addition to the birth weight also the length at birth is retarded (14). However our patient No. 1 was of normal length at birth but later he



For Case 2 The right leg is shorter and thinner than the left



Fig 3 Case 1 Absence of the toes on the left foot

presented a distinctly reduced increase in length and is now below the 3rd percentile.

The retarded birth length follows the patient throughout the growth period and remains usually below the 3rd percentile for normal subjects as opposed to premature infants (1). Generally the growth curve runs approximately parallel with the normal curve (14).

The development of the ossification centres can be normal (13) but in the majority of cases it is retarded (2, 15) as compared with the age of the patient although in most cases it corresponds to the growth in length. Occasionally the development of the ossification centres can be more retarded on the affected than on the unaffected side (3, 12). Mowley *et al.* (8) described two patients with accelerated

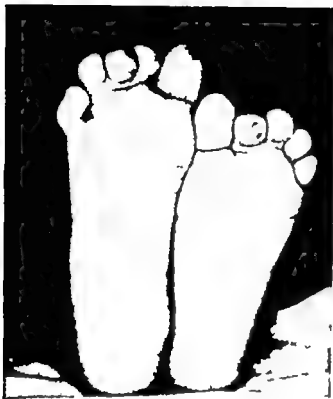


Fig. 1 Case 1 Short left foot

Delivery was normal. The patient was born at term but the birth weight was only 1320 g and the length 40 cm. The placenta was normal. The toes on the left foot were missing (Fig. 3). The right leg was distinctly thinner and shorter than the left. At the age of 9 months the weight was 4380 g and the height 61 cm. Motor development was retarded but mental development normal. The right leg was 1 cm shorter than the left (Fig. 4). The right arm was also shorter than the left and the right side of the face slightly flattened. There was increased tendency to perspiration.

On examination at the age of 18 months normal urinary excretion was found of pituitary gonadotrophins, 17 ketosteroids, 17 ketogenic steroids and estrogens. Chromosomes were normal. Urinary excretion of amino acids normal. EEG normal. X-ray of skull and chest normal. X-ray of the feet showed absence of the second and third phalanges of the fourth and fifth toes on the right. On the left side all phalanges were missing.

At the age of 18 months the ossification centres were found to be retarded corresponding to the age of 3-6 months but identical on both sides. At the age of 15 months distinct estrogenization of the vaginal mucosa was demonstrated.

Since then the patient has been followed up in our out-patient clinic and has presented acceleration of the weight increase and almost normal rate of increase in height. Hence at the age of 2 years the height is 75 cm and the weight 7500 g.

## DISCUSSION

The first four symptoms are sometimes described as the major symptoms the others as minor symptoms. According to Reister & Scherz (9) three major symptoms will be sufficient to establish the diagnosis and hence both our patients fulfil the criteria for the diagnosis of Silver's syndrome.

Occasionally the asymmetry is described as hemihypertrophy but, as stated by Ferner et al (3) the term asymmetry is to be preferred, since it might be difficult to decide whether a genuine hemihypertrophy is present, or whether it is a question of a corresponding hemihypotrophy.

The asymmetry can vary greatly from mild and localized cases to severe total asymmetry (14). Mild cases might escape recognition at birth but will become evident during growth. The asymmetry can be unilateral or crossed (15). Since the asymmetry might occasionally be very slight there is nothing to prevent us



Fig. 2 Case 1 The left leg is shorter and thinner than the right

- 11 Russell A A syndrome of intra uterine dwarfism recognizable at birth with crinio-facial dysmorphism disproportionately short arms and other anomalies *Proc Roy Soc Med* 47 1040 1954
- 12 Schenmacher G & Niederhoff H Das Syndrom nach Russell und Silver *Helv Paediat Acta* 22 404 1967
- 13 Silver H K Klyne W George J & Denner W C Syndrome of congenital hemihypertrophy shortness of stature and elevated urinary gonadotrophins *Pediatrics* 11 368 1953
- 14 Silver H K Asymmetry short stature and variations in sexual development *Amer J Dis Child* 107 495 1964
- 15 Stool S & Cohen P Silver's syndrome *Amer J Dis Child* 105 199 1962
- 16 Szalay G C Intrauterine growth retardation versus Silver's syndrome *J Pediatr* 64 234 1964
- 17 Tanner J M & Ham T J Low birthweight dwarfism with asymmetry (Silver's syndrome) Treatment with human growth hormone *Arch Dis Child* 44 235 1969

Submitted Jan 5 1970

Accepted Jan 10 1970

Dept of Paediatrics  
Apmisgshuset  
Glostrup  
Denmark

**Key words** Silver's syndrome congenital hemihypertrophy growth retardation

ed development of the ossification centres but these two patients were 11 and 15 years old, respectively; and possibly this might explain the accelerated bone development. Experience with respect to the course during adolescence in patients with Silver's syndrome is still too limited to decide whether in all patients the development of the ossification centres will accelerate during adolescence. According to Silver (14) early sexual development can be demonstrated in about one third of the patients but in spite of this feature no acceleration in the rate of increase in length occurs. The youngest patient hitherto observed presenting increased urinary excretion of gonadotrophins was 17 months old (2). In our Case 2 the excretion of gonadotrophins was normal but vaginal secretion showed estrogenization as early as the age of 15 months. Ferrer et al (4) described estrogenization of vaginal smears in two children without asymmetry who in other respects fulfilled the criteria for the diagnosis of Silver's syndrome.

The incidence of minor symptoms varies considerably and similar changes are frequently observed in the ordinary types of hemihypertrophy (10).

Mental development can be retarded in Silver's syndrome (3) but is normal in most cases. Both our patients presented increased tendency to perspiration but as in the case described by Girard & Kaufmann (6) we have not been able to detect any metabolic cause of this phenomenon.

The etiology is completely unknown and may be multifarious. Chromosome studies in most cases reveal normal conditions. However Hook & Yunis (7) described one case with 18 trisomy and Ferrer et al (3) one case with diploid-triploid mosaicism. Familial occurrence or sex differences have not been demonstrated (14). Growth hormone studies have been made in a few cases only (6, 17) and in one case only was reduction found (17).

There is no possibility of treating the asymmetry. Only in very isolated cases have attempts been made to treat the growth retardation with

growth hormone (17). During this treatment the growth in length increased considerably but the normal level was not reached in spite of treatment for 1 to 3 years. In patients with normal production of growth hormone this effect might indicate that the patient's own growth hormone is not normal or that the patient does not react adequately to a normal production of growth hormone. However experience with growth hormone treatment in Silver's syndrome is too sparse as yet to permit an evaluation of the effect.

### SUMMARY

A description is given of a boy and a girl with asymmetry, shortness of stature and low birth weight despite being born at term. The girl showed estrogenization of the vaginal mucosa at the age of 15 months. No chromosomal abnormalities were demonstrated.

### REFERENCES

1. Black J. Low birth weight dwarfism. *Arch Dis Child* 36: 633, 1961.
2. Curi J, F J, Vanucci R, C Grossman H & New M. Elevated serum gonadotrophins in Silver's syndrome. *Amer J Dis Child* 114: 651, 1967.
3. Ferrer F, Ferrer S, Stalder G, Buhler E, Brumatter F & Klein D. Congenital asymmetry associated with diploid triploid mosaicism and large satellites. *Lancet* i: 110, 1964.
4. Ferrer P, Shepard T H & South E L. Growth disturbances and values for hormone excretion in various forms of precocious sexual development. *Pediatrics* 38: 258, 1961.
5. Fraumeni J F Jr, Geyer C F & Manning A D. Wilms' tumor and congenital hemihypertrophy. *Pediatrics* 40: 886, 1967.
6. Girard J & Kaufmann H J. Der Russel Zwerg. *Mischr Kinderheilk* 113: 696, 1965.
7. Hook E B & Yunis J J. Congenital asymmetry associated with trisomy 18 mosaicism. *Amer J Dis Child* 110: 551, 1963.
8. Moseley J B, Moloshok R E & Freiburger R H. The Silver syndrome. Congenital asymmetry, short stature and variations in sexual development. *Amer J Roentgen* 97: 74, 1966.
9. Reiter H C & Scherz R G. Silver syndrome. *Amer J Dis Child* 107: 410, 1964.
10. Ringrose R E, Jabbour J I & Keck D K. Hemihypertrophy. *Pediatrics* 46: 434, 1965.

the summer of 1968. This shows that poliomyelitis has in fact not been eradicated.

The incidence of polio in Finland after the intense vaccination with inactivated poliovaccine in the years 1960-62 showed a sudden drop. Only 2 virologically diagnosed cases have occurred since both had contracted the infection abroad. In 6 other suspected cases the diagnosis could not be confirmed virologically.

In spite of this satisfactory epidemiological situation a survey of the present vaccination program and the immunity status of the population shows that some risk of a decrease in the immunity of the population is at hand.

To avoid incidents like the one in Poland it is suggested that the immunity status of the population be surveyed at intervals and that search for enteroviruses from sewage should be continued.

Vaccination of children and revaccination of young women should be intensified. The young men already receive a booster in military service. Finally live vaccine should be available for emergency.

#### Discussion

*R. Tammilehto* Live poliomyelitis vaccine is now in use in most countries but in Finland and Sweden inactivated vaccine is still utilized. Vaccination with live vaccine would probably result in 4-5 cases of poliomyelitis annually. This risk would be worth taking only if the annual number of poliomyelitis cases would exceed this number.

*P. Halonen* Current position of measles vaccines

In general Finnish pediatricians have assumed a very cautious attitude to measles vaccinations. This may be partly caused by the mild nature of measles in Finland and the great decline in number of deaths caused by this disease over a 30-year period. In 1939 412 deaths were reported out of 55 000 measles cases (7 deaths/1 000 notified cases). During last 5 years the

mean annual incidence has been about 20 000 and the number of deaths 3 (0.15 death/1 000 notified cases). The last figure is almost identical with measles mortality in England (0.2 death/1 000 notified cases). However the precise incidence of complications, especially of postinfectious encephalitis and of late neurological disorders such as SSPE and multiple sclerosis (?) is not known. Another reason for cautious attitude is the possibility that vaccinations will postpone the disease 10 to 20 years resulting in very severe forms of measles in adults. This problem however can be well avoided by revaccinations if necessary.

The experience with inactivated measles vaccines has been disappointing, the potency being low. In addition children immunized with inactivated vaccines and later exposed to natural measles have developed measles with atypical skin reactions and severe pneumonia indicating hypersensitivity to measles virus.

With live further attenuated measles virus vaccines a huge experience has accumulated. Approximately 40 million doses of vaccines have been used in the USA and other countries. The protective efficacy has been clearly demonstrated by the dramatic decline in measles in the USA, the figures for epidemiological years 1967-68 and 1968-69 being 10% or less of the prevaccination average. The serological tests indicate that immunity should last long, if not as long as after disease. HI antibody response in vaccinees resembles that of measles patients though after vaccination the titres decrease slightly faster during 6-7 years than after natural disease.

The reactions caused by live measles vaccines depend on the attenuation level. No gamma globulin is given with the more attenuated strains. A higher fever and a modified rash occurs only occasionally with these vaccines. Interest has recently focused in more severe illnesses such as encephalitis associated with measles vaccinations. In the USA the rate of neurological manifestations has been approximately 1 out of 200 000 vaccinees compared to 1/400 or 1/1000 after natural measles and in no



## PROCEEDINGS OF PAEDIATRIC SOCIETIES

### THE FINNISH PAEDIATRIC SOCIETY

Meeting Oct 18 1969

(together with the Finnish Society for the study of Infectious Diseases)

#### *K Penttinen Prophylaxis of epidemic influenza by vaccinations*

In spite of vaccines available against epidemic influenza the disease shows the same epidemic patterns as before. In Scandinavian countries the number of doses of vaccines used in the beginning of the Hong Kong epidemic were the following: Sweden 1 100 000, Finland 200 000, Denmark and Norway 100 000. The ineffectiveness of the prophylactic vaccinations is partly due to the nonavailability of vaccines when a new strain appears and partly to the low efficiency rate of vaccines available. At best the protection rate of influenza vaccines is 2-3 and often less. Monovalent Hong Kong strain vaccine in Finland only gave a 20-30% protection (L. Haapanen unpublished observation). The data available from other countries are equally poor. The influenza vaccines have the lowest efficiency of all vaccines in use. They are not recommended for general unrestricted use but only for medical risk groups according to the judgement of a physician. In such a group the effectiveness of vaccination (0-50%) is not critical; everything possible must be done. Another group which might be considered is medical personnel who take care of severely ill patients.

The reasons for the low efficiency are now becoming clear. Influenza is a surface infection without a viremic stage. Antibodies in serum are of minor importance as compared with local IgA. The decision to use or not to use

influenza vaccines is not much influenced by the complications of the vaccination. Theoretically there are many unfavorable possibilities, but the use of millions of doses without reported serious complications has tranquilized the manufacturers and vaccinators. The use of vaccines with only one or two components seems reasonable because of the possible competition between the similar antigens. The best vaccination schedules should be worked out. A new approach is the use of purified particle vaccines which probably can be injected or inhaled.

### DISCUSSION

*Lisa Haapanen* The staff of the Aurora hospital was vaccinated in 1968 with a single dose of monovalent Hong Kong strain vaccine. Comparison with a control group showed no statistically proved protection. Neither was the prophylactic use of Amantadine effective.

*N Oker Blom* In medical students a 60-70% protection was obtained with Amantadine.

#### *N Oker Blom Vaccination against polio*

Poliomyelitis has practically disappeared from most of the countries in the Western hemisphere thanks to intense vaccination programs. In Poland about 600 cases of poliomyelitis occurred in the Poznan region within a few months in

PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY

Meeting in Interlaken September 15 1969

J Schmitz & J Rey (Paris) *Characteristics of glycyl-L-leucine and glycyl-L-valine dipeptidase activities in the human intestine*

The hydrolysis of two very similar substrates (glycyl-L-leucine and glycyl-L-valine) by an homogenate of human intestinal mucosa was studied to prove the possibility of the hydrolysis of several peptides by one enzyme. pH optimum (7.7 and 8.0), best inactivation temperature (50 and 53°C) and apparent  $K_m$  (0.03 M/l) of the two activities using Josephson & Lindberg's assay method were appraised.

The very similar results suggest that both activities take place at one site. However, using a Technicon Autoanalyzer with a short column (30 cm filled with type XX 8 60-0 Phoenix r sm) it was shown that there is no competitive inhibition between the two substrates since glycyl-L-leucine reduces the activity of glycyl-L-valine dipeptidase to half without modifying its apparent  $K_m$ . This unexpected result was corroborated by the lack of influence of a mixture of glycine and leucine on the hydrolysis of glycyl-L-valine, implying that this non-competitive inhibition is not related to the liberation of amino-acids. Hence it was of interest to investigate the possible influence on glycyl-L-valine dipeptidase activity of a dipeptide very probably not hydrolysed by the same enzyme as glycyl-L-valine and glycyl-L-leucine. For this purpose, alanyl-L-proline was chosen and it was found that alanyl-L-proline too reduces it (though less than glycyl-L-leucine) the hydrolysis of glycyl-L-valine without modification of the apparent  $K_m$ .

Further studies are necessary to clarify and ascertain these first results. Anyhow, the "one site" hypothesis for these two activities must be reconsidered.

P Kuitunen, K. Krohn & J. K. Visakorpi (Helsinki) *Gastric mucosal changes and antibodies to intrinsic factor and to the parietal cells in some endocrine disorders in childhood*

The occurrence of gastric atrophy and pernicious anaemia in hypoparathyroidism is a well known syndrome especially in adults. Pernicious anaemia combined with gastric atrophy may occur in some other endocrine disorders such as thyroid diseases and Addison's disease.

The gastric mucosa, its function, the absorption of vit. B<sub>12</sub> and the antibodies to intrinsic factor (according to Ardeman and Chamarrin) and to parietal cells (indirect immunofluorescence) were investigated in 9 hypoparathyroid children (3 1/2 - 16 1/2 years) and 20 children (9-18 1/2 years) with autoimmune thyroiditis.

Three of 9 children with idiopathic hypoparathyroidism had achlorhydria. Two of them also had pernicious anaemia, gastric atrophy and antibodies to parietal cells and to intrinsic factor. These patients also had steatorrhea despite a normal jejunal mucosa and one of them had Addison's disease as well. The third achlorhydric patient had a borderline Schilling test and partial villous atrophy of the jejunal mucosa but a normal gastric mucosa. In the other hypoparathyroid patients gastric studies were normal except in two who were hypo-

case has a relationship between the encephalitis or death and the vaccination been definitely proved. This rather small indirect evidence of more severe complications occurring after natural measles has shifted the selection of vaccine strains to more attenuated ones.

There is no further laboratory clinical or epidemiological data to be expected on which the decision to use a vaccine in Finland could be based. Commencement of vaccination in one or two health districts with gradual increase to cover the whole country seems to be a reasonable approach in our country. The practical steps depend now on the activity of health officers and on the interest of pediatricians rather than on virologists.

### Discussion

*A. Penttinen* Is the frequency of SSPE higher after natural measles than after vaccination against measles?

*P. Halonen* Nobody knows!

### M. Valle *Vaccination against smallpox*

In spite of world wide vaccination programmes about 80 000 cases of smallpox are still recorded annually. During the past 20 years there have been about 80 outbreaks of the disease in the smallpox free countries. The great increase in air travel has increased the risk that smallpox could be brought into our country. It is thus necessary continuously to control the vaccination certificates of passengers arriving

from smallpox countries and from outbreak areas. Two features of smallpox outbreaks in smallpox free countries should be emphasized. First the primary case is most probably modified which means that the diagnosis may be difficult especially if the possibility of smallpox is not kept in mind. Secondly smallpox spreads mainly by close contact and the risk of transmission is very high in hospitals. Thus hospital personnel should be properly vaccinated annually.

As vaccination complications occur more often in patients who have certain allergic disease are on glucocorticoid therapy or are pregnant these should not be vaccinated. When these people travel to another smallpox free country vaccination against smallpox should not be required. If such individuals have to be vaccinated during smallpox outbreaks or when travelling to endemic areas antivaccinal gamma globulin should be given simultaneously. To lower the risk of postvaccination encephalitis primary vaccination is recommended to be performed at the age of about one year.

In Finland a liquid calf lymph vaccine is used. The preparation of freeze dried smallpox vaccine which will be donated to WHO has been started. This will also be available on request for vaccinations in our country. The multiple puncture (with a bifurcated needle) or the multiple pressure techniques are the vaccination methods recommended in Finland.

It is emphasized that the vaccination reaction should be observed and all complications reported.

Meeting Dec 13 1969

### *Juvenile use of narcotics*

#### Panel Discussion

Participants N. Hallman MD chairman J. Seise municipal attorney J. Idanpaan Heikkilä

MD Pirkko Idanpaan Heikkilä MD E. Varilo MD and R. Järkkölä MA

*E. I. Wallgren*

technique was used using as substrate an esterified mixture of natural oils and trimyristine in order to obtain as closely as possible the same percentage of six fatty acids: lauric, myristic, palmitic, stearic, oleic and linoleic distributed at random on the glycerol molecules. The triglycerides were isolated on a silicic acid column after heat inactivation of the lipase. Lipid extraction by a system of ether, heptane, ethanol and water and saponification of free fatty acids.

The relative composition of recovered triglycerides is the same as that of ingested oil for fatty acids of more than 12 carbon atoms (index of recovery  $\approx 1$ ); the percentage of lauric acid on the other hand is significantly diminished (index of recovery  $0.5 \pm 0.2$ ). The results confirm that the rate of hydrolysis of fatty acids of 14-18 carbon atoms is independent of the chain length and the degree of saturation. This does however not imply that pancreatic lipase has a particular affinity for lauric acid; the findings may also be explained by the fact that the fatty acid is more soluble in the aqueous phase and that saponification from positions  $\beta$  to  $\alpha$  of the glycerol molecule is more rapid when lauric acid is compared with longer fatty acids.

† Plenet U, Spahn & E. Petrich (Jena)  
*Serum lipids in the newborn and young in famt—Influence of nutrition*

There is a typical serum lipid pattern of the newborn immediately after birth which is roughly characterized by a very low level of all lipid fractions and a remarkably small quantity of linoleic acid. Already in the course of the first few days a rapid shift of the serum lipid pattern can be observed and it takes only a short time till the serum lipids level up to the values found in later infancy. These changes are dependent on the regime of nutrition especially on the quantity of dietary fat. Our own recent studies were concentrated on the question: are other factors than the dietary fat in-

take correlated with the postnatal changes of serum cholesterol and does the intake of food cholesterol influence the value of serum cholesterol? Though the total cholesterol increased in the first days of life the cholesterol linoleate was found to decrease while the other ester fractions especially oleate and the esters of tri and tetraenes showed a marked elevation. There was no evidence that the percentage of esterified cholesterol was affected essentially by immaturity at birth or by hyperbilirubinaemia. During nutrition with human milk an immediate higher rate of cholesterol linoleate ester synthesis in the second and third week of life can be expected while the concentrations of tri and tetraene esters decrease. Likewise beyond the postnatal period no influence of body weight, degree of maturation at birth or different course of postnatal period could be registered. Our findings support the conclusion that alimentary factors prevail in the development of serum lipid pattern from birth to later infancy.

There is another difference in the level of serum cholesterol correlated to the regime of nutrition. In accordance with other investigations there was a considerable rise of total serum cholesterol in infants fed human milk. In contrast total serum cholesterol was significantly lower in infants fed modified cow's milk. These adapted formulas are characterized by replacement of butterfat by vegetable oils rich in polyenoic acids. The lower levels of serum cholesterol in infants fed adapted formulas have been attributed to the depressing effect on serum cholesterol of an high intake of polyenoic acids. This hypothesis has not been proved and it lacks some sound evidence as the intake of polyenoic acids with human milk is far from low. Recently it has been shown in adults that the intake of dietary cholesterol may influence the serum cholesterol level significantly. Our own findings suggest that the intake of dietary cholesterol is relatively high in infants fed human milk and considerably lower in infants fed adapted formulas filled with vegetable oils.

chlorhydric. Elevated immunoglobulins was mostly due to raised IgG. The patients with idiopathic hypoparathyroidism seemed first to suffer from hypoparathyroidism conjoined with Addison's disease and later from gastric atrophy conjoined with pernicious anaemia.

In autoimmune thyroiditis achlorhydria was found in 3 patients. Two of these had gastric atrophy and one had atrophic gastritis grade 1-2. Five more patients had superficial or intestinal gastritis but no abnormalities in gastric function. Pernicious anaemia was found in only 1 case with gastric atrophy. Antibodies to parietal cells were found in 5 of 8 patients with and in 3 of 11 patients without gastric mucosal changes. In autoimmune thyroiditis concomitant disorders were found: diabetes mellitus in 3, malabsorption syndrome with jejunal villous atrophy in 3, lack of immunoglobulin A in 2 and rheumatoid arthritis combined with nephropathy in 1 patient.

The results of this study demonstrate that even in childhood pernicious anaemia occurs rather frequently in conjunction with endocrinopathies. Our findings indicate an immunologic mechanism which perhaps continues for several years.

G Zoppi (Ferrara), D H Shmerling, D Garburro & A Prader (Zurich). *The electrolyte and protein contents and outputs in duodenal juice after pancreozymin and secretin stimulation in normal children and in patients with cystic fibrosis*

The duodenal contents and the secretion of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{HCO}_3^-$  and total protein were studied after pancreozymin and secretin stimulation in 12 healthy control children and in 5 patients with cystic fibrosis of the pancreas (CF). Duodenal contents were collected through a double balloon triple lumen duodenal tube thus avoiding contamination by gastric juice and distal losses.

In the controls the following secretion rates were found (means  $\pm 1$  SD, mg/kg/50 min after IV stimulation):  $\text{Na}^+$   $13.9 \pm 6.4$ ,  $\text{K}^+$   $0.8 \pm 0.3$

$\text{Ca}^{++}$   $0.12 \pm 0.05$ ,  $\text{Mg}^{++}$   $0.05 \pm 0.01$ ,  $\text{HCO}_3^-$   $15 \pm 7$ , protein  $15.4 \pm 5.5$ , volume  $3.9 \pm 1.6$  ml/kg/50 min.

In CF patients decreased secretion rates were found:  $\text{Na}^+$   $2.5 \pm 0.3$ ,  $\text{K}^+$   $0.2 \pm 0.07$ ,  $\text{Ca}^{++}$   $0.04 \pm 0.02$ ,  $\text{Mg}^{++}$   $0.03 \pm 0.02$ ,  $\text{HCO}_3^-$   $1.4 \pm 1.5$ , protein  $9.7 \pm 8.8$ , volume  $0.8 \pm 0.4$  ml/kg/50 min. The ratio  $\text{Na}^+$  to  $\text{K}^+$  secretion were found to be 17.4 in the controls and 12.5 in CF patients and the ratio  $\text{Ca}^{++}$ /protein 0.008 in the controls and 0.004 in CF.

Secretion velocity expressed in  $\mu\text{g/kg/min}$ , of the electrolytes and of protein was analyzed separately for the post pancreozymin and the post secretin periods i.e. for the acinar and the tubular secretion phases. In normal controls the secretion velocities of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$  and  $\text{HCO}_3^-$  were higher after secretin stimulation whereas those of  $\text{Ca}^{++}$  and protein were higher after pancreozymin stimulation. In CF patients  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$  and  $\text{HCO}_3^-$  secretion velocities after secretion were somewhat lower than after pancreozymin and the values for  $\text{Ca}^{++}$  and protein did not show any significant change. Compared to the values in normals the secretion velocities of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$  and  $\text{HCO}_3^-$  in CF patients are more depressed after secretin than after pancreozymin stimulation whereas  $\text{Ca}^{++}$  and protein were more affected during the latter phase. The authors discuss the possible role of a disturbance of the tubular secretion of ions and of the acinar production of Ca protein complexes in the genesis of symptoms in this disease.

C Ricour & J Rey (Paris). *Hydrolysis rates of long chain triglycerides. Study in vivo*

The fatty acid composition of triglycerides remaining in the intestinal lumen during fat digestion was analyzed to study the relation between the rates of hydrolysis of triglycerides and the chain length and the degree of saturation of the fatty acids. After intubation of first jejunal loop with a single lumen tube the test meal

persisted in 3 patients and in Case 5 the intestinal mucosa and the disaccharidase returned normal when tested 3 months after the institution of therapy. Clinical improvement in all cases was striking. We believe that this mode of therapy was life saving in these 5 infants.

Jon (Paris) *Immunohistochemical study of the intestinal mucosa in children*

An immunohistochemical study of the intestinal mucosa has been performed in 37 children comprising controls (9), coeliac disease (17), cow's milk intolerance (7), intestinal lymphangiectasia (1) and gammaglobulin deficiency (1 lymphocyte), 1 Bruton disease and 1 "acquired" hypogammaglobulinemia.

Duodeno-jejunal biopsies were obtained with the Crosby capsule and were immediately frozen. Sections 6  $\mu$  thick were cut in a cryostat at  $-22^{\circ}\text{C}$  and after fixation in absolute methanol they were incubated with fluoresceinated antisera. The slides were examined and photographed under ultraviolet light using a dark field condenser. The specificity of the antisera (anti IgA, IgM and IgG immunoglobulin) was checked by immunoelectrophoresis and by double immunodiffusion in Ouchterlony plates.

The results demonstrate that the lamina propria of intestinal mucosa in normal children contains as in adults large numbers of IgA type plasma cells. The amount of the IgM and especially the IgG type cells is much smaller.

In untreated coeliac disease the number of fluorescent cells using IgA immunoglobulin antiserum is always increased and inconstantly so using anti IgM. This cellular hyperplasia disappears when gluten is omitted from the diet for several months. Cellular hyperplasia reappears a few weeks after reintroducing gluten in the diet of the patients. Corresponding changes are observed in cow's milk intolerance.

In primary antibody deficiency states (Bruton disease "acquired" hypogammaglobulinemia lymphocyte  $\alpha$ ) no immunoglobulin-containing cells are found in the lamina propria of intestinal

mucosa. In secondary types such as lymphangiectasia or malabsorption syndromes the number and the distribution of the IgA-containing cells are normal or increased. No case with malabsorption or mucosal alterations and isolated IgA-deficiency was observed.

These immunofluorescent studies appear useful to investigate the changes in immunoglobulin synthesis associated with gastrointestinal disorders and to obtain accurate information about the nature of the cellular hyperplasia of the lamina propria seen in malabsorption syndromes.

S. North & G. Moscatelli (Trieste) *Studies on intestinal mucosa and PHA stimulated lymphocytes in malabsorption syndrome and immunologic deficiencies of children*

The authors take once more into consideration the results of a research on a few enzyme activities of intestinal mucosa and the PHA responsiveness of lymphocytes.

A statistically significant increase of intestinal beta-glucuronidase is chiefly emphasized in malabsorption syndromes. It is the first time that an increase instead of a decrease of an enzyme is observed in this syndrome. Since beta glucuronidase is a lysosomal enzyme the role of lysosomes in the pathogenesis of malabsorption syndrome might be taken into consideration. In fact an increase of lysosomal like bodies of epithelial cells of intestinal mucosa was demonstrated by Shiner and the authors. But in interpreting hyperactivity of beta glucuronidase immuno-competent lymphoid cells of intestinal mucosa cannot be forgotten. PHA stimulation of in vitro cultivated lymphocytes provokes in control subjects not only blast transformation but an increase of beta glucuronidase too. In 1 case only of malabsorption syndrome was blast transformation reduced while in 2 other cases betaglucohydrolase of PHA stimulated lymphocytes increased in a lesser degree. Similar results were obtained in a case of a gamma globulinemia and in a case

D Nussle J Frei C Bozic & E Gautier (Lausanne) *Proximal and distal lesions in malabsorptive states. A morphological enzymatic and functional study*

Twenty children with coeliac disease aged 6 months - 15 years were investigated

In order to assess the extension of the lesions a proximal and a distal jejunal biopsy was obtained, using the Crosby capsule. The distal biopsy was taken 120-150 cm below the ligament of Treitz. A small mercury bag was used to propel the capsule and metoclopramide was given in repeated doses. The mucosal specimens were examined under a dissecting microscope as well as histologically. Enzyme activities of lactase, sucrase, maltase and alkaline phosphatase were measured. Furthermore, balance studies and loading tests were performed in the patients.

Similar to the great variation in the severity of the clinical manifestations of coeliac disease we found great variations between patients in the extent of the morphological lesions and in the impairment of the enzyme activities.

A close relationship between the extension of the morphological lesion and the degree of the malabsorption did not exist, but steatorrhea was regularly found if the distal mucosa showed a partial or subtotal atrophy.

A better correlation was found between the overall lactase activity, as estimated from the sum of proximal and distal lactase activities and the tolerance to lactose in these patients. Intolerance to lactose occurs only if the lactase activity at both sites is markedly decreased.

There is no fixed relationship between the morphology and the enzyme activities especially not in the presence of moderate morphological alterations of the distal mucosa.

H Shwachman R M Filler & K T Khaw (Boston, Mass.) *A new method of treating malnourished infants with severe chronic diarrhoea*

A newly described technique of total parenteral nutrition devised by Wilmore & Dudrick has

been used in our institution over the past year with some modifications. A detailed description of the method is presented in the *New England Journal of Medicine* 281: 589-594, 1969. The details of the preparation and composition of the infusate, its placement and proper care and the routine clinical measurements on some of the patients studied will be found in the article mentioned.

This method has been used in the treatment of 5 infants with severe malnutrition secondary to chronic diarrhoea who failed to respond to the usual medical measures including I.V. therapy. Four of these patients were treated at other hospitals without success prior to their transfer to our hospital. These infants had a similar history with onset of diarrhoea and vomiting shortly after birth, inability to tolerate formula (and many were tried), no fever except in 1 patient with a diagnosis of otitis media. Many laboratory tests were performed all with a variety of negative results. The following table lists the age, the life line was instituted, the duration of this therapy and the weight of the infant at the onset and at the end of the parenteral therapy.

	Age at start (days)	Duration (days)	Total administration weight kg	
			start	end
Patient 1	56	44	2.9	3.8
2	65	39	3.3	4.5
3	150	52+	4.0	5.5
4	140	51	3.8	5.5
5	77	30	3.6	4.8

Oral feedings were instituted gradually first with clear solutions and the initial formula given was a special preparation prepared by Mead Johnson Co (3200AD) (Dr Herbert Sirtti) which was composed of glucose, MCT, casein hydrolysate, starch plus vitamins and minerals.

Serial intestinal biopsies were performed in 4 infants. At the beginning of the special therapy on 3 infants and on several subsequent occasions. Histologic changes were noted in all cases with Grade 2 to 3 villous atrophy. There was also a reduction in activity of lactase, maltase and sucrase. This secondary deficiency

Alexandra Hospital for Children have been studied. The following descriptive terms have been used "Fingers" described narrow villi with parallel sides. Broader villi were divided into narrow "fingers" and thin ridges depending on their width. Short thick ridges described a mucosa where villi were replaced by thickened ridges sometimes having a convoluted appearance.

There were 46 neonates, 39 children between 1 month and 1 year and 21 over 1 year of age. Fifty nine of the 106 children had evidence of gastroenterological disease.

Thin ridges, leaves and tongues were most often seen in the duodenum and proximal jejunum of the non gastroenterological group with fingers and narrow leaves in the ileum. The gastroenterological group often had short thick ridges in the proximal gut. These changes were most marked on the tops of the plicae circulares where at times the mucosa was almost flat. Tongues and narrow leaves were more frequent than fingers in the ileum of these children. However the chief differences between the two groups occurred in the proximal small bowel and this was the region of the severest abnormality although in some the whole small intestine was involved. Apart from a higher percentage of neonates having finger like villi in the ileum there did not appear to be a significant difference of appearances in relation to age.

M. Gracey, V. Burke & Ch. M. Anderson  
(Birmingham) *Malabsorption of carbohydrate associated with abnormal intestinal flora*

In the blind loop syndrome steatorrhea and deconjugation of bile salts are related to the presence of abnormal bacterial flora within the small bowel. Disturbances of fat and vitamin B<sub>12</sub> absorption and of protein metabolism have previously been demonstrated in this syndrome. However carbohydrate malabsorption occurring in the presence of bacterial overgrowth in the small bowel has not been generally recognized.

We have demonstrated impairment of absorption of carbohydrate in 8 babies with abnormal intestinal bacterial flora. All patients presented with severe watery diarrhoea and dehydration following the ingestion of glucose, galactose or fructose.

The patients are considered in two clinical groups:

1. Four newborns presenting following surgery for the relief of small bowel obstruction.
2. Four babies presenting following an illness resembling gastroenteritis.

Carbohydrate malabsorption was determined semi quantitatively by using the ClinTest method to estimate concentration of stool sugar.

In all patients there was an abnormality of intestinal flora (mainly overgrowth of *E. coli*) which paralleled the severity of symptoms. Unconjugated bile salts were present in samples of intestinal juice from all patients while mono saccharide malabsorption was present. Small bowel biopsy in the nonsurgical group showed normal histology and disaccharidase activity. In the surgical group dilatation of the proximal intestinal segment results in stasis; however barium studies showed no anatomical abnormalities in the non surgical patient.

The mechanism of production of this form of carbohydrate malabsorption which involves the active transport of glucose and galactose and the more passive transport of fructose is not presently known but is under study.

C. Borroni, F. Dagna, S. Carelli, L. Massimo, A. Fossati, Guglielmoni & P. Durand (Genoa) *Lymphocyte transformation in milk allergy*

Blast transformation of lymphocytes from peripheral blood in the presence of milk proteins proved the clinical diagnosis of milk allergy in 2 children, 1 of them suffering from hypodermic anaemia from chronic intestinal bleeding, the other one from urticaria and colicapses (Borroni *et al* *Min. Ped.* 21: 1070, 1969).



with severe hypogammaglobulinemia. In these subjects beta glucuronidase was also reduced in intestinal mucosa.

B Strandvik, R Zetterstrom & A Norman (Stockholm) *Malabsorption during the course of cholestasis of infancy*

The results of studies on 6 infants with cholestasis were presented. Two infants with intrahepatic cholestasis were found to have an impairment of fat absorption as marked as in 2 infants with extrahepatic biliary atresia. The degree of steatorrhea was found to be correlated to the impairment of the excretion of bile acids into the intestine.

Follow up studies of 4 infants with intrahepatic cholestasis demonstrated that intestinal bile acid excretion and consequently the absorption of triglycerides and of vitamin A were impaired as long as jaundice persisted. Bile acid excretion into the intestine as well as the absorption of fat and vitamin A were found to normalize in the 2 patients in whom hyperbilirubinemia disappeared.

J H van de Kamer (Zeist) & H A Weyers (Utrecht) *Lactic acid in faeces*

The estimation of the lactic acid content of faeces is of use in the diagnosis of disturbed carbohydrate absorption.

It is a drawback that the faecal lactic acid content measured is dependent on the laboratory method used. With the method of Long both L and D lactic acid are determined whereas the enzymatic method using L lactate dehydrogenase measures L lactic acid only. As Long's method is not entirely specific for total lactic acid it is incorrect to determine D lactic acid content indirectly by the difference between the two estimations. However total lactic acid content i.e. the sum of L and D lactic acid may be determined specifically by gas chromatography so that the faecal D lactic acid

may be accurately determined by the difference between the gas chromatographically determined L- and D lactic acid and the enzymatically measured L lactic acid.

Application of these methods demonstrates the following:

1 The faeces of breastfed infants sometimes contain only L lactic acid and sometimes besides L much D lactic acid.

2 The faeces of most cases of disaccharidase deficiency contained only L lactic acid.

3 A lactulose tolerance test performed on healthy infant resulted in L lactic acid only in the faeces.

4 The faeces of a patient with protein loss enteropathy contained no lactic acid after alteration of the gut flora with Moxiform however lactic acid was present. This was shown to be L lactic acid. As lactic acid in this case could not have been derived from dietary carbohydrates it is suggested that it arose from breakdown products of mucus.

J A Walker Smith (Sydney Australia) *Small bowel morphology in infancy and childhood. A dissecting microscope study of 106 autopsies*

Little is known of normal small bowel morphology in children nor the extent of mucosal changes demonstrable on biopsy along the small intestine. A post mortem study would extend such knowledge, but autolysis of the epithelium prevents adequate histological study. If the autolysed surface cells of post mortem bowel are washed off the connective tissue cores of the villi are exposed and the overall arrangement of the villi and crypts can be studied using the dissecting microscope. That the appearances seen are comparable to fresh biopsy specimens has been demonstrated by allowing autolysis of the epithelium of 14 biopsies to occur and then examining these in the same manner.

Specimens from the duodenum proximal jejunum and distal ileum taken from 106 childhood autopsies performed at the Royal

Hooft *et al* of the methionine malabsorption syndrome (*Ann Paediat* 205 73 1965)

F Severi G Rondini S Zaverio & M Bruschelli (Pavia) *Prolonged neonatal hyperbilirubinaemia and pregnane 3(Alpha) 20(Beta) Diol in maternal milk*

We carried out the determination of pregnane 3 $\alpha$  20 $\beta$ -diol (a) in milk of mothers whose infants showed prolonged neonatal unconjugated hyperbilirubinaemia and (b) in milk of unselected mothers whose newborns were tested for bilirubinaemia in the first weeks of life

(a) 7 breast fed infants with prolonged jaundice were observed for a period of 9 months. Their ages ranged from 8 to 60 days and their total bilirubinaemia from 3.8 to 22.6 mg/100 ml. The direct reacting bilirubin was always very low (5-8% of the total). In all cases the determination of pregnane 3 $\alpha$  20 $\beta$  diol in maternal milk was performed. The compound was present in 5 cases in 2 it was not. In these 2 cases the newborns were 8 days and 26 days old the serum bilirubin was respectively 21.4 and 10 mg/100 ml. In neither case other causes of prolonged hyperbilirubinaemia were demonstrable. The bilirubinaemia fell quickly to normal values after discontinuing breast feeding.

The administration of milk containing pregnane 3 $\alpha$  20 $\beta$  diol to a non jaundiced newborn aged 5 days for a period of 11 days did not cause a rise of serum bilirubin.

(b) A systematic determination of pregnane 3 $\alpha$  20 $\beta$ -diol was performed in 17 samples of milk obtained from 17 mothers. The compound was present in the milk of three mothers and their newborns showed prolonged unconjugated hyperbilirubinaemia.

On the basis of our investigation the presence of pregnane 3 $\alpha$  20 $\beta$ -diol in maternal milk seems quite frequent. We found the compound in addition to 5 selected cases (with prolonged jaundice) in 3 out of 17 unselected samples of milk. When the compound was present in maternal milk we always observed prolonged jaun-

dice. On the other hand in 2 cases of prolonged jaundice we were not able to demonstrate pregnane 3 $\alpha$  20 $\beta$ -diol in maternal milk.

G Flatz (Chuan Mai Thailand) *Lactose intolerance in Thailand*

Intolerance to dietary lactose due to deficiency of intestinal lactase is widespread in populations of African origin. Recently an even higher incidence has been reported in small groups of oriental students in USA and Australia. Our own study (lactose tolerance tests in over 200 individuals from northern and central Thailand) shows that lactose intolerance has a very high incidence in healthy adults. In children the ability to digest lactose seems to disappear between the ages of 2 and 4 years. With respect to the question of an adaptive or hereditary etiology of lactose intolerance we found that adults who took cow's milk for periods up to 3 years did not develop lactose tolerance. Several children in northern Thailand who had taken milk regularly since weaning were also intolerant to lactose. Tolerance tests in offspring of marriages between Southeast Asians and Europeans show a clear dimorphism and the results do not seem to correspond to the dietary history. The results point at a genetic origin of lactose intolerance rather than an adaptive mechanism.

C Polonovski J Badoual & J Navarro (Paris) *Congenital and familial digestive polyintolerance to proteins including human milk*

Two siblings with chronic multiple intolerance to dietary proteins including human milk proteins studied in collaboration with P Grenet are described.

Frequent vomiting chronic diarrhoea and failure to thrive were noted from birth in Case 1. At the age of 25 days fat droplets were noted in the stools later steatorrhea and diarrhoea disappeared. Although gluten had not

The positive test consisted of a remarkable increase of transformed lymphocytes after a 120 hour blood culture in the presence of 0.5 mg/ml milk proteins, in comparison with a control blood culture without antigen in some cases cultures with the milk fractions, lactoalbumin and  $\beta_1$  lactoglobulin were carried out in addition. Immunologic lymphocyte response was proved with a PHA stimulation in all cases. We judged the positive result as the expression of an immunologic lymphocyte response to the antigen milk-proteins. Beside the morphologic evaluation we considered the presence of a high number of mitoses obtained after a 3 hours incubation with Colcemid the presence of labelled cells and chromosomes with tritiated thymidine added to the culture in S phase the increase of beta glucuronidase in positive cultures lysosome enzyme which increases in the lymphocyte cultures added with PHA.

We examined 39 children with this test. In 11 controls of the same age as the patients there was no difference between the two cultures one with milk proteins and the other one without. Also 4 children with coeliac disease were negative. In 21 infants (4 to 12 months) suffering from chronic diarrhoea which seemed to be related with the milk feeding the results achieved were positive in 10 children and negative in 11. In 3 children suspected to suffer from milk allergy the response has been positive in one of them the test showed to be strongly positive to  $\beta_1$  lactoglobulin. The substitution of powdered milk with sterilized and boiled milk caused a complete normalization of the infant.

In the positive cases we found an increase of the lymphoblast index ranging from 4 to 20% in the cultures with milk proteins. In 2 children examined during the acute phase of the disease the spontaneous blast transformation was higher than in all the other cases which finding was probably due to the antigenic stimulation *in vivo*.

In comparison with several tests commonly used for the diagnosis of milk allergy the lymphoblastic transformation with milk pro-

teins shows to be the more specific one. Relying on our experience we propose this test for the diagnosis of cow's milk allergy.

Ch S Bartsocas (Athens) J D Crawford & S O Thier (Boston Mass.) *Demonstration of an independent transport system for L-methionine in rat intestine and kidney*

The irregular elevation of urinary methionine excretion despite large losses of the other neutral amino acids in patients with Hartnup disease suggested to Efron (*New Eng J Med* 272:1058-1107, 1965) that methionine transport may be dependent upon an enzyme system different from that determining transport of the other neutral amino acids.

In order to test this hypothesis L-methionine transport was studied by measuring uptake of the amino acid in rat kidney and intestine slices *in vitro*. Accumulation of L-methionine against a chemical concentration gradient was shown. The transport was found to be a saturable process in that the rates of accumulation failed to increase at high medium concentrations. Its dependence in both tissues upon oxidative metabolism was evident from the inhibition of uptake under anaerobic conditions. 2,4-dinitrophenol, however, inhibited only the kidney accumulation. Transport was found to be relatively pH insensitive but critically dependent upon the presence of sodium. Normal rates of methionine accumulation were restored when tissue was replaced in sodium containing medium after incubation in sodium free sucrose but were subnormal after incubation in sodium free Tris medium. Of all amino acids tested in competitive inhibition experiments only methionine and possibly L-valine were found to inhibit its uptake.

These results show that L-methionine transport depends upon an enzyme system independent of the system(s) responsible for transport of the other neutral amino acids. The findings are compatible both with Efron's hypothesis and with the subsequent description by

Hooft *et al* of the methionine malabsorption syndrome (*Ann Paediatr* 203 73 1965)

dice on the other hand in 2 cases of prolonged jaundice we were not able to demonstrate pregnane 3 $\alpha$  20 $\beta$  diol in maternal milk

F Seven G Rondini ■ Zavento & M Bruschi (Pavia) *Prolonged neonatal hyperbilirubinaemia and pregnane 3(Alpha) 20(Beta) Diol in maternal milk*

We carried out the determination of pregnane 3 $\alpha$  20 $\beta$  diol (a) in milk of mothers whose infants showed prolonged neonatal unconjugated hyperbilirubinaemia and (b) in milk of unselected mothers whose newborns were tested for bilirubinaemia in the first weeks of life

(a) 7 breast fed infants with prolonged jaundice were observed for a period of 9 months. Their ages ranged from 8 to 60 days and their total bilirubinaemia from 3.8 to 22.6 mg/100 ml. The direct reacting bilirubin was always very low (5-8% of the total). In all cases the determination of pregnane 3 $\alpha$  20 $\beta$  diol in maternal milk was performed. The compound was present in 5 cases in 2 it was not. In these 2 cases the newborns were 8 days and 26 days old the serum bilirubin was respectively 21.4 and 10 mg/100 ml. In neither case other causes of prolonged hyperbilirubinaemia were demonstrable. The bilirubinaemia fell quickly to normal values after discontinuing breast feeding.

The administration of milk containing pregnane 3 $\alpha$  20 $\beta$  diol to a non jaundiced newborn aged 5 days for a period of 11 days did not cause a rise of serum bilirubin.

(b) A systematic determination of pregnane 3 $\alpha$  20 $\beta$  diol was performed in 17 samples of milk obtained from 17 mothers. The compound was present in the milk of three mothers and their newborns showed prolonged unconjugated hyperbilirubinaemia.

On the basis of our investigation the presence of pregnane 3 20 $\beta$  diol in maternal milk seems quite frequent. We found the compound in addition to 5 selected cases (with prolonged jaundice) in 3 out of 17 unselected samples of milk. When the compound was present in maternal milk we always observed prolonged jaun-

G Flatz (Chuan Mai Thailand) *Lactose intolerance in Thailand*

Intolerance to dietary lactose due to deficiency of intestinal lactase is wide spread in populations of African origin. Recently an even higher incidence has been reported in small groups of oriental students in USA and Australia. Our own study (lactose tolerance tests in over 200 individuals from northern and central Thailand) shows that lactose intolerance has a very high incidence in healthy adults. In children the ability to digest lactose seems to disappear between the ages of 2 and 4 years. With respect to the question of an adaptive or hereditary etiology of lactose intolerance we found that adults who took cow's milk for periods up to 3 years did not develop lactose tolerance. Several children in northern Thailand who had taken milk regularly since weaning were also intolerant to lactose. Tolerance tests in offspring of marriages between Southeast Asians and Europeans show a clear dimorphism and the results do not seem to correspond to the dietary history. The results point at a genetic origin of lactose intolerance rather than an adaptive mechanism.

C Polonovski J Badoual & J Navarro (Paris) *Congenital and familial digestive polyintolerance to proteins including human milk*

Two siblings with chronic multiple intolerance to dietary proteins including human milk proteins studied in collaboration with P Grenet are described.

Frequent vomiting, chronic diarrhoea and failure to thrive were noted from birth in Case 1. At the age of 25 days fat droplets were noted in the stools, later steatorrhea and diarrhoea disappeared. Although gluten had not

The positive test consisted of a remarkable increase of transformed lymphocytes after a 120 hour blood culture in the presence of 0.5 mg/ml milk proteins, in comparison with a control blood culture without antigen in some cases cultures with the milk fractions lactoalbumin and  $\beta_1$  lactoglobulin were carried out in addition. Immunologic lymphocyte response was proved with a PHA stimulation in all cases. We judged the positive result as the expression of an immunologic lymphocyte response to the antigen milk proteins. Beside the morphologic evaluation we considered the presence of a high number of mitoses obtained after 73 hours incubation with Colcemid the presence of labelled cells and chromosomes with tritiated thymidine added to the culture in S-phase the increase of beta glucuronidase in positive cultures lysosome enzyme which increases in the lymphocyte cultures added with PHA.

We examined 39 children with this test. In 11 controls of the same age as the patients there was no difference between the two cultures one with milk proteins and the other one without. Also 4 children with coeliac disease were negative. In 21 infants (4 to 12 months) suffering from chronic diarrhoea which seemed to be related with the milk feeding the results achieved were positive in 10 children and negative in 11. In 3 children suspected to suffer from milk allergy the response has been positive in one of them the test showed to be strongly positive to  $\beta_1$  lactoglobulin. The substitution of powdered milk with sterilized and boiled milk caused a complete normalization of the infant.

In the positive cases we found an increase of the lymphoblast index ranging from 4 to 20% in the cultures with milk proteins. In 2 children examined during the acute phase of the disease the spontaneous blast transformation was higher than in all the other cases which finding was probably due to the antigenic stimulation *in vivo*.

In comparison with several tests commonly used for the diagnosis of milk allergy the lymphoblastic transformation with milk pro-

teins shows to be the more specific one. Relying on our experience we propose this test for the diagnosis of cow's milk allergy.

Ch. S. Bartsocas (Athens), J. D. Crawford & S. O. Thier (Boston Mass.) *Demonstration of an independent transport system for L methionine in rat intestine and kidney*

The irregular elevation of urinary methionine excretion despite large losses of the other neutral amino acids in patients with Hartnup disease suggested to Elfron (*New Eng J Med* 272:1058-1107, 1965) that methionine transport may be dependent upon an enzyme system different from that determining transport of the other neutral amino acids.

In order to test this hypothesis L methionine transport was studied by measuring uptake of the amino acid in rat kidney and intestine slices *in vitro*. Accumulation of L methionine against a chemical concentration gradient was shown. The transport was found to be a saturable process in that the rates of accumulation failed to increase at high medium concentrations. Its dependence in both tissues upon oxidative metabolism was evident from the inhibition of uptake under anaerobic conditions. 2,4 dinitrophenol however inhibited only the kidney accumulation. Transport was found to be relatively pH insensitive but critically dependent upon the presence of sodium. Normal rates of methionine accumulation were restored when tissue was replaced in sodium containing medium after incubation in sodium free sucrose but were subnormal after incubation in sodium free Tris medium. Of all amino acids tested in competitive inhibition experiments only ethionine and possibly L valine were found to inhibit its uptake.

These results show that L methionine transport depends upon an enzyme system independent of the system(s) responsible for transport of the other neutral amino acids. The findings are compatible both with Elfron's hypothesis and with the subsequent description by

normal child or according to the age it is only a little increased when compared with normal values in the literature (3). There is no significant correlation either with the intestinal albumin loss or with the intensity of the clinical picture. This seems to indicate that the albumin synthesis is nearly at its maximum in normal subjects and that there is only a little compensation possible. In case of enteric albumin loss the organism tries at first to keep a normal albuminemia first by decreasing the metabolic catabolism of albumin and secondly by displacing the albumin equilibrium from the extra to the intravascular pool. hypoalbuminemia appears only when a critical threshold is reached making any compensation mechanism no longer possible.

## REFERENCES

1. Waldmann T A, Wochner R D & Strober W. The role of the gastro-intestinal tract in plasma protein metabolism. *Am J Med* 46: 275, 1969.
2. Kerr R M, Du Bort J J & Holt P R. Use of  $^{125}\text{I}$  and  $\text{C}^{14}$  labeled albumin for the measurements of gastrointestinal and total albumin catabolism. *J Clin Invest* 45: 2064, 1967.
3. Krasilnikoff R A, Andersen S B & Rossm N. Albumin metabolism in normal children. *Proceedings of Biological Fluids*, vol. 14. Elsevier Publishing Company, Amsterdam, 1966.

O Sazl, O Podhradská & J Tichý (Brno). *Gastrointestinal findings in Lowe's syndrome*.

Ever since Lowe and co-workers described the oculo-cerebrorenal syndrome much attention has been paid especially to the renal but also to the ocular and cerebral symptoms though no author has to the best of our knowledge ever mentioned gastro-intestinal disturbances besides anorexia, vomiting and constipation. Only lately Bartoocas and co-workers described a defect in intestinal amino acid transport in Lowe's syndrome similar to those in cystinuria, Hartnup disease and familial iminoaciduria.

In our patient we saw anemia and failure to thrive for long periods even at times when he had almost no protein loss in the urine. In

analogy with similar clinical pictures in the nephrotic syndrome where we had found protein loss from the gastrointestinal mucosa we anticipated these symptoms in our patient. We could confirm protein loss by repeated Congo-red tests and by other methods quantitatively also by estimating the Brounphenol blue positive substances.

The malabsorption was confirmed by the existence of steatorrhea. The xylose test was twice positive, the proline test once positive, the lipiodol test once negative and once strongly positive.

The histology of the small intestine showed atrophic changes of degree 1-2.

As to the electronmicroscopic investigation the brush border of the enterocytes was nearly normal only seldom showing slight changes resembling those in coeliac disease and in Down's syndrome. Rather important changes were seen in the goblet cells which were only 1/4 their normal size, very scarce, about 1/5 their normal number and degenerated.

Treatment with penicillamins seemed to be of some value. Should the findings be confirmed in more such patients it is suggested to name this syndrome the oculo-cerebro-renal intestinal syndrome.

P Peltkonen & J K Viskorpi (Helsinki). *Precipitins to cow's milk and gluten and the serum level of IgA in the follow up of coeliac patients*.

The study was undertaken to determine whether during the later course of coeliac disease changes in the precipitating antibodies and in the serum level of IgA might provide information about the effects of the dietary treatment and its discontinuation. Forty infants and children with coeliac disease were followed up for a minimum of 2 years. A double diffusion technique and a single radial immunodiffusion method were used.

During treatment with a gluten free diet antibodies to cow's milk disappeared in only half of the patients whereas antibodies to gluten

been introduced in the child's diet, abdominal distension was present. Pancreatic insufficiency,  $\alpha$ - $\beta$  lipoproteinemia, disaccharidase deficiency, chloride diarrhoea and exsudative enteropathia were excluded by appropriate tests. During various dietary trials it was noted that meat was badly tolerated. A soy formula, however, was better tolerated but weight gain was inconsistent. Human milk was not well tolerated and failure to gain weight persisted. During 17 months diarrhoea alternated with constipation including an episode of intestinal obstruction caused by carrots. The possibility of Hirschsprung's disease was definitely excluded by rectal biopsy. There were slight biochemical signs of malabsorption. Nevertheless there was no steatorrhea at this age. Partial villous atrophy explained these symptoms. The stools were always alkaline with a high fecal ammonia. Amino acid chromatography of the stools revealed no selective amino acid malabsorption. Moreover there was no post prandial rise of blood ammonia which excluded the protein intolerance described by Perheentupa & Visa-Korpi.

A synthetic diet consisting of amino acids and a standard commercial protein hydrolysate was now given and this resulted in a rapid improvement of all symptoms and in weight gain. Reintroduction of beef or horse meat, cow's milk or ovalbumine was followed by immediate weight loss, vomitings and in some instances diarrhoea. Reintroduction of human milk caused the same symptoms. The lactose of human milk had previously been removed by dialysis and the fat content had been reduced by centrifugation. Using four different substrates no deficiency of dipeptidase was found in a jejunal biopsy specimen.

Serum antibodies to lactoglobulin at the titer of 1/128 but no antibodies against maternal milk proteins were detected. The lymphocyte transformation test was difficult to interpret.

The brother of Case 1 was 12 years at time of investigation and he had shown similar symptoms with vomiting, failure to thrive and diar-

rhoea already when he was breast fed. Subsequently cow's milk and meat were also badly tolerated. During his first year he presented eczema. Within some years protein intolerance improved and meat could be given without harmful effects at 10 years.

The early onset and the familial incidence suggest a genetic origin. The parents are first cousins and there is eczema and asthma in the family.

It seems that this disease is a separate allergic syndrome.

The bad tolerance to human milk in which lactose and most of the lipids had been removed, was unequivocally demonstrated. This distinguishes the congenital disorder of our patients from classical milk protein allergy which may be associated with intolerance to other nutritive proteins as previously described.

H. Gaze & A. Donath (Berne) *Determination of intestinal loss and turnover of albumin in children with coeliac disease*

In a 14 month old child without gastrointestinal disease and in 4 children aged between 6 and 20 months presenting the clinical picture and the morphological alterations of intestinal mucosa typical for coeliac disease the turnover and enteric loss of albumin were studied. All cases were normoproteinemic and had normal serum albumin. The method was a modification of that described by Waldmann (1) and Kerr *et al.* (2) using the simultaneous intravenous injection of two preparations of human serum albumin, one labeled with  $^{125}\text{I}$  and the other with  $^{51}\text{Cr}$ . The method and the results are compared with those of other authors.

The results show that in coeliac disease the enteric loss of albumin is always 2 to 4 times higher than in the normal subject and correlates with the gravity of the clinical picture. On the other hand the younger the child, the greater is the relative plasma volume. The same applies to the albumin degradation rate: however in coeliac disease it stays within the range of the

normal child or according to the age it is only a little increased when compared with normal values in the literature (3). There is no significant correlation either with the intestinal albumin loss or with the intensity of the clinical picture. This seems to indicate that the albumin synthesis is nearly at its maximum in normal subjects and that there is only a little compensation possible. In case of enteric albumin loss the organism tries at first to keep a normal albuminemia first by decreasing the metabolic catabolism of albumin and secondly by displacing the albumin equilibrium from the extra- to the intravascular pool hypoalbuminemia appears only when a critical threshold is reached making any compensation mechanism no longer possible.

## REFERENCES

1. Wahlsten T. A. Wochoer R. D. & Strober W. The role of the gastro-intestinal tract in plasma protein metabolism. *Am J Med* 46: 275 1969.
2. Herr R. V. De Boer J. J. & Hobb P. R. Use of  $^{125}$ I and  $^{147}$ Sm labeled albumin for the measurement of gastrointestinal and total albumin catabolism. *J Clin Invest* 46: 2064 1967.
3. Krasilnikoff R. A. Andersen S. B. & Rosen N. Albumin metabolism in normal children. *Proceedings of Biological Fluids* vol. 14. Elsevier Publishing Company Amsterdam 1966.

## O. Saxl, O. Podhradská & J. Tachy (Brno) Gastrointestinal findings in Lowe's syndrome

Ever since Lowe and co-workers described the oculo-cerebrorenal syndrome much attention has been paid especially to the renal but also to the ocular and cerebral symptoms though no author has to the best of our knowledge ever mentioned gastro-intestinal disturbances besides anorexia, vomiting and constipation. Only lately Bartoocas and co-workers described a defect in intestinal amino acid transport in Lowe's syndrome similar to those in cystinuria Hartnup disease and familial aminoaciduria.

In our patient we saw anemia and failure to thrive for long periods even at times when he had almost no protein loss in the urine. In

analogy with similar clinical pictures in the nephrotic syndrome where we had found protein loss from the gastrointestinal mucosa we anticipated these symptoms in our patient. We could confirm protein loss by repeated Congo-red tests and by other methods quantitatively also by estimating the Bromphenol blue positive substances.

The malabsorption was confirmed by the existence of steatorrhea. The xylose test was twice positive, the proline test once positive, the lipodol test once negative and once strongly positive.

The histology of the small intestine showed atrophic changes of degree 1-2.

As to the electronmicroscopic investigation the brush border of the enterocytes was nearly normal, only seldom showing slight changes resembling those in celiac disease and in Down's syndrome. Rather important changes were seen in the goblet cells which were only 1/4 their normal size, very scarce about 1/5 their normal number and degenerated.

Treatment with penicillamine seemed to be of some value. Should the findings be confirmed in more such patients it is suggested to name this syndrome the oculo-cerebro-renal intestinal syndrome.

## P. Pellkonen & J. K. Viikari (Helsinki) Pre- cipitins to cow's milk and gluten and the serum level of IgA in the follow up of coeliac patients

The study was undertaken to determine whether during the later course of coeliac disease changes in the precipitating antibodies and in the serum level of IgA might provide information about the effects of the dietary treatment and its discontinuation. Forty infants and children with coeliac disease were followed up for a minimum of 2 years. A double diffusion technique and a single radial immuno-diffusion method were used.

During treatment with a gluten free diet antibodies to cow's milk disappeared in only half of the patients whereas antibodies to gluten



been introduced in the child's diet abdominal distension was present. Pancreatic insufficiency  $\alpha$ - $\beta$  lipoproteinemia disaccharidase deficiency chloride diarrhoea and exsudative enteropathia were excluded by appropriate tests. During various dietary trials it was noted that meat was badly tolerated. A soy formula, however, was better tolerated but weight gain was inconsistent. Human milk was not well tolerated and failure to gain weight persisted. During 17 months diarrhoea alternated with constipation including an episode of intestinal obstruction caused by carrots. The possibility of Hirschsprung's disease was definitely excluded by rectal biopsy. There were slight biochemical signs of malabsorption. Nevertheless there was no steatorrhea at this age. Partial villous atrophy explained these symptoms. The stools were always alkaline with a high fecal ammonia. Amino acid chromatography of the stools revealed no selective amino acid malabsorption. Moreover there was no post prandial rise of blood ammonia which excluded the protein intolerance described by Perheentupa & Visa korpä.

A synthetic diet consisting of amino acids and a standard commercial protein hydrolysate was now given and this resulted in a rapid improvement of all symptoms and in weight gain. Reintroduction of beef or horse meat cows milk or ovalbumine was followed by immediate weight loss vomitings and in some instances diarrhoea. Reintroduction of human milk caused the same symptoms. The lactose of human milk had previously been removed by dialysis and the fat content had been reduced by centrifugation. Using four different substrates no deficiency of dipeptidase was found in a jejunal biopsy specimen.

Serum antibodies to lactoglobulin at the titer of 1/128 but no antibodies against maternal milk proteins were detected. The lymphocyte transformation test was difficult to interpret.

The brother of Case 1 was 12 years at time of investigation and he had shown similar symptoms with vomiting failure to thrive and diar-

rhoea already when he was breast fed. Subsequently cow's milk and meat were also badly tolerated. During his first year he presented eczema. Within some years protein intolerance improved and meat could be given without harmful effects at 10 years.

The early onset and the familial incidence suggest a genetic origin. The parents are first cousins, and there is eczema and asthma in the family.

It seems that this disease is a separate allergic syndrome.

The bad tolerance to human milk, in which lactose and most of the lipids had been removed was unequivocally demonstrated. This distinguishes the congenital disorder of our patients from classical milk protein allergy which may be associated with intolerance to other nutritive proteins as previously described.

#### H. Gaze & A. Donath (Berne) *Determination of intestinal loss and turnover of albumin in children with coeliac disease*

In a 14 month old child without gastrointestinal disease and in 4 children aged between 6 and 20 months presenting the clinical picture and the morphological alterations of intestinal mucosa typical for coeliac disease the turnover and enteric loss of albumin were studied. All cases were normoproteinemic and had normal serum albumin. The method was a modification of that described by Waldmann (1) and Kerr *et al* (2) using the simultaneous intravenous injection of two preparations of human serum albumin one labeled with  $^{131}\text{I}$  and the other with  $^{51}\text{Cr}$ . The method and the results are compared with those of other authors.

The results show that in coeliac disease the enteric loss of albumin is always 2 to 4 times higher than in the normal subject and correlates with the gravity of the clinical picture. On the other hand the younger the child the greater is the relative plasma volume. The same applies to the albumin degradation rate however in coeliac disease it stays with

(a) determination of faecal amine content in ulcerative colitis

(b) measurement of the low molecular weight fatty acids in the faeces in cases of protein losing enteropathy and small intestinal resection

(c) bacterial counts in combination with low molecular weight fatty acids estimation and faecal lactic acid content determination in a case of protein losing enteropathy

2 Bacterial metabolites absorbed in the colon and excreted in the urine. The bacterial metabolites of tryptophan which appeared in the urine in a case of tryptophan malabsorption were described

3 Once the presence of protein malabsorption has been established one must investigate possible aetiological factors. In this connection the determination of peptidase activity in gut mucosa biopsy specimens in a case of coeliac disease and a case of generalized malabsorption syndrome due to mucopolysaccharidosis was reported

#### B Lindquist (Lund) *Sugar induced diarrhoea*

Two main types of sugar induced diarrhoea exist: (1) deficiency of disaccharidase activities; (2) deficiency of the transport systems for monosaccharides. This is however more or less an artificial division as there is a close relationship between digestion and absorption of sugars: these processes should rather be regarded as two steps of one common function.

During recent decades there has been some discussion whether sugar induced diarrhoea is mainly of osmotic or fermentative nature. Although both mechanisms may exist it could be concluded from the considerable amounts of sugar excreted with faeces in patients with glucose-galactose malabsorption that the diarrhoea is mainly of osmotic origin. This has been confirmed by Launila in intubation studies in patients with lactose malabsorption: the unabsorbed disaccharide (lactose) caused a considerable movement of water into the intestinal lumen.

Next to the pathophysiology of the disturbed absorption. Glucose tolerance tests as well as intubation studies have shown that a small but definite absorption of glucose occurs in glucose-galactose malabsorption: in upper jejunum it amounts to about 10%. Under normal conditions galactose has been found to be absorbed slightly faster than glucose when these sugars are given separately. In glucose-galactose malabsorption the opposite is true: on oral sugar loading tests the fecal excretion of galactose was found to be roughly 2½ times that of glucose indicating that in this condition the cells of the intestinal mucosa are almost impermeable to galactose.

A most interesting question is whether we are dealing with a defect bound to the intestinal mucosa or a more generalized disturbance. Studies in our department have shown that in cases of glucose-galactose malabsorption there are signs of a disturbed intermediate carbohydrate metabolism. This seems however to be related to a secondary carbohydrate deprivation rather than to the basic disease itself: it is also reversible. The same phenomenon was observed in a malnourished patient with cystic fibrosis of the pancreas.

Although lactase deficiency may be an exception, the acquired forms of sugar malabsorption are probably secondary to unspecific lesions of the intestinal mucosa. The nature of such a lesion and in what way such a lesion affects the mucosa is however still unclear. During recent years this problem has become actualized as there have been described several cases of so-called intractable diarrhoea in early infancy in which all kinds of monosaccharides are slowly absorbed. In one case observed by us an intestinal biopsy performed in the acute phase showed in certain areas partly destroyed villi and villi of somewhat irregular shape and furthermore an increased number of inflammatory cells, especially plasma cells were observed. The diarrhoea was interpreted as caused by an unspecific lesion of the intestinal mucosa primarily inflammatory changes due to an enteric infection but with subsequent damage of

nearly always disappeared during the first few months. The initially elevated serum level of IgA normalized rapidly. Thirteen patients were followed up beyond reinstitution of a gluten-containing diet. In ten of these recurrence of the mucosal lesion was verified within 2 to 10 months and this was accompanied by symptoms in 6 cases. Significant elevation of the IgA level ensued in 6 of the 10 patients and precipitins to cow's milk and gluten reappeared in 4 and 8 patients respectively. Only one of the relapsers showed none of these signs. In 3 patients the small intestinal mucosa remained normal. Nevertheless one of these reacted to the change of diet with an elevation of the

IgA level and in another precipitins to gluten were again detectable.

Determination of the serum IgA level and precipitating antibodies to cow's milk and gluten is of value in the follow up of coeliac patients. Disappearance of antibodies may be a favourable sign indicative of normalization of the mucosal lesion. Persistence or reappearance of antibodies to gluten during a gluten free diet is indicative of non adherence to the diet. After discontinuation of the diet reappearance of antibodies and/or elevation of the IgA level is strongly suggestive of a relapse of the mucosal lesion which is not, however always detected by these tests.

## EUROPEAN SOCIETY FOR PAEDIATRIC RESEARCH

### EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY

Joint Meeting in Interlaken September 16 1969

J. H. van der Kamer (Zeist) & H. A. Weijers (Utrecht) *The diagnosis of protein malabsorption*

Dietary protein digestion is facilitated by heating during the process of preparation prior to consumption. Heat denatures the proteins thus facilitating subsequent enzymatic breakdown.

Proteolysis of dietary protein in the stomach results from the action of pepsine HCl: the bonds split being mostly those of phenylalanine, leucine and tyrosine. Of the pancreatic enzymes trypsin splits the bonds with lysine and arginine, chymotrypsin those with tyrosine, phenylalanine and tryptophane and the carboxypeptidases A and B split the bonds with carboxyl terminal amino acids. Proteolysis is completed in the intestinal mucosal cell by carboxypeptidase A, amino peptidase, prolinase, prolidase, dipeptidase and probably other as yet unrecognized enzymes.

Disturbances which may occur are disturbed activation of the above named enzymes, disturbed gastro intestinal motility, disturbed innervation and/or perfusion of the gastro intestinal tract and disturbed intracellular function. Small intestinal resection, the blind loop syndrome and protein losing enteropathy in addition will lead to abnormal protein digestion. This will all lead to protein malabsorption resulting in an excess of peptides reaching the ileocaecal region leading to an increase of the bacterial flora and disturbing the normal bacterial pattern. The result is production of excessively large quantities of normal and also abnormal bacterial metabolites and toxins as well as osmotic changes within the gut lumen.

Diagnostic investigation will therefore have to be focused on

1. The gut flora and its metabolites in the faeces. The following examples were discussed

kinase a proteolytic enzyme secreted by the mucosa of the small intestine. Enterokinase converts the proenzyme of trypsin (trypsinogen) into active trypsin. trypsin then activates the other pancreatic zymogens (chymotrypsinogens and procarboxypeptidases).

Thus enterokinase is the key enzyme in this process and enterokinase deficiency must result in a complete failure to activate pancreatic zymogens.

So far 2 cases of intestinal enterokinase deficiency are known (1, 2). In both patients the disease manifested as a severe disturbance of protein digestion with diarrhoea from birth, failure to thrive, anaemia and severe hypoproteinaemia.

The biochemical evidence that the primary defect of protein digestion in these patients is due to enterokinase deficiency is provided by three observations:

1. In the duodenal fluid the activities of the proteolytic enzymes trypsin, chymotrypsin and carboxypeptidase are undetectable or extremely low. The addition of enterokinase to the fluid *in vitro* results in a rapid appearance of normal activities of all three enzymes. From this it must be concluded that the pancreatic zymogens are secreted normally into the duodenum but cannot be activated.

2. After Gel filtration of duodenal fluid enterokinase activity can be directly measured. It is undetectable in the patients' duodenal fluid.

3. No enterokinase activity is detected in the patients' duodenal mucosa obtained by peroral biopsy.

Enterokinase deficiency is probably a congenital deficiency of one single intestinal enzyme. Evidence that it is a primary defect rather than the effect of a more generalized small bowel disease is provided by the following observation. At the age of 17 months one of the patients was reinvestigated. At this time she had following a substitution therapy with pancreatic extracts containing enterokinase fully recovered from the secondary effects of protein malabsorption. A duodenal biopsy was taken which was morphologically normal and

had normal lactase activity. Enterokinase activity however was still undetectable in the mucosal homogenate.

## REFERENCES

1. Hadorn B, Tarlow M J, Lloyd I L & Wolf O H. *Lancet* 1: 813, 1969.
2. Polonovski C, Hadorn B & Baer H. Unpublished observation.

M J Tarlow (London), B Hadorn (Berne) & M W Arthurton (Bradford). *Intestinal enterokinase deficiency. Report of 2 cases*.

Two patients with intestinal enterokinase deficiency have been studied. Both were girls who presented with diarrhoea from birth and failure to thrive. Only one of the children developed hypoproteinaemia. Investigations showed absence of proteolytic activity in the duodenal juice. chymotryptic and carboxypeptidase activity appeared after the addition of trypsin. The addition of a small amount of enterokinase to the juice resulted in rapid and complete activation of pancreatic proteolytic enzymes. normal levels of trypsin, chymotrypsin and carboxypeptidase appeared in the duodenal juice showing that the precursor forms of all these enzymes were present. In the first patient enterokinase activity was assayed after gel filtration of the juice on a Sephadex G 100 column and was not detected. enterokinase activity was similarly absent in the soluble fraction of a homogenized duodenal biopsy specimen although it was present in control mucosal biopsies from patients with coeliac disease. Duodenal biopsies from both children were normal to light and electron microscopy and had normal disaccharidase activities.

Both children had steatorrhoea in early infancy but as their general nutritional state improved fat absorption became normal. Despite the absence of proteolytic activity in the duodenal juice faecal trypsin was present in normal concentrations. In both children treatment with oral pancreatic extract resulted in prompt control of symptoms and in improved growth.

the absorption mechanisms causing sugar as well as protein intolerance

*G Meeuwisse (Lund) Renal glucosuria in glucose-galactose malabsorption*

The glucosuria of 3 fasting patients with glucose-galactose malabsorption was measured quantitatively with a sensitive method for the detection of glucose in urine. The concentration of glucose was in the range in which Christux gives varying results (15–40 mg/100 ml) or sometimes slightly higher. This corresponded to a moderately increased excretion of glucose per unit time which was not found in four of the parents of the patients. Two of the patients were subjected to forced diuresis by drinking large quantities of water. The glucose concentration fell to values as low as 3 mg/100 ml which was however higher than in controls with diuresis of the same magnitude. The maximal reabsorption capacity of glucose ( $T_{mG}$ ) was found to be normal in 3 patients and before the attainment of  $T_{mG}$  there was not much increase of the glucosuria.

The patients are probably capable of transporting glucose actively in the renal tubuli by a mechanism other than the intestinal mucosa. It was speculated that in normal individuals the mechanism responsible for the bulk transport of glucose in the small intestine has some task, but only a minor one in the kidney.

*T Lindberg (Malmö) & H Karlsson (Lund) Developmental changes in intestinal dipeptidases of foetal and neonatal pig as related to changes in the ultrastructure of the mucosal cells*

The dipeptidase activities (units/mg N) on L-alanyl L-glutamic acid, L-alanyl L-proline and glycyl L-leucine are very low in porcine intestinal mucosa from early foetal development (2 cm C-R length). The activities increase uniformly up to the stage of 14 cm C-R length. The L-alanyl L-proline dipeptidase activity thereafter

remains at about the same level throughout the whole developmental period. The other two activities however increase considerably from the stage of 14 cm C-R length. The values registered in newborn piglets not fed with colostrum are 2–3 times higher than those from slaughtered pigs. However, these activities decrease abruptly and significantly after the newborn pig has ingested colostrum.

The electron microscopic studies reveal a striking parallelism between enzyme levels registered and appearance of microvilli in the absorptive cells. Microvilli are practically lacking in fetuses of 2 cm C-R length but begin to differentiate in the 4–5 cm fetuses. With proceeding foetal development they reach step by step the appearance earlier noted in the mucosa of newborn piglets (Mattsson & Karlsson, 1966).

The results obtained complete and illustrate earlier findings in man and rat (Lindberg, 1966). Correlation between structure of mucosal cells and magnitude of dipeptidase activity thus exists in rat (Lindberg & Öwman, 1966) and pig. Moreover, in man it has been shown that intestinal dipeptidase activities are significantly reduced in coeliac disease (Lindberg *et al*, 1968) where the structure of the mucosal cells, i.e. the microvilli, are known to be affected.

The decrease of the dipeptidase activities after colostrum ingestion cannot yet be satisfactorily explained. Experiments performed *in vitro* did not demonstrate any direct inhibitory effect of porcine colostrum on the activities, while other experiments *in vivo* have shown that the decrease is connected in one way or another with the ingestion of colostrum.

*H Hadorn (Berne) Intestinal enterokinase deficiency. A newly recognized disorder of protein digestion in early infancy*

Proteolytic enzymes of the pancreas are stored in the gland as inactive precursors (zymogens). They become activated only after the pancreatic secretion has reached the duodenum. The key enzyme in this activation process is entero-

kinase a proteolytic enzyme secreted by the mucosa of the small intestine. Enterokinase converts the proenzyme of trypsin (trypsinogen) into active trypsin. trypsin then activates the other pancreatic zymogens (chymotrypsinogens and procarboxypeptidases).

Thus enterokinase is the key enzyme in this process and enterokinase deficiency must result in a complete failure to activate pancreatic zymogens.

So far 2 cases of intestinal enterokinase deficiency are known (1, 2). In both patients the disease manifested as a severe disturbance of protein digestion with diarrhoea from birth, failure to thrive, anaemia and severe hypoproteinaemia.

The biochemical evidence that the primary defect of protein digestion in these patients is due to enterokinase deficiency is provided by three observations:

1. In the duodenal fluid the activities of the proteolytic enzymes trypsin, chymotrypsin and carboxypeptidase are undetectable or extremely low. The addition of enterokinase to the fluid *in vitro* results in a rapid appearance of normal activities of all three enzymes. From this it must be concluded that the pancreatic zymogens are secreted normally into the duodenum but cannot be activated.

2. After Gel filtration of duodenal fluid enterokinase activity can be directly measured. It is undetectable in the patients' duodenal fluid.

3. No enterokinase activity is detected in the patients' duodenal mucosa obtained by peroral biopsy.

Enterokinase deficiency is probably a congenital deficiency of one single intestinal enzyme. Evidence that it is a primary defect rather than the effect of a more generalized small bowel disease is provided by the following observation. At the age of 17 months one of the patients was reinvestigated. At this time she had following a substitution therapy with pancreatic extracts containing enterokinase fully recovered from the secondary effects of protein malabsorption. A duodenal biopsy was taken which was morphologically normal and

had normal lactase activity. Enterokinase activity however was still undetectable in the mucosal homogenate.

## REFERENCES

1. Hadorn B, Tarlow M J, Lloyd J A & Wolff G H. *Lancet* 1: 813, 1969.
2. Polonowski C, Hadorn B & Buer H. Unpublished observation.

M J Tarlow (London), B Hadorn (Berne) & M W Arthurton (Bradford). *Intestinal enterokinase deficiency. Report of 2 cases*.

Two patients with intestinal enterokinase deficiency have been studied. Both were girls who presented with diarrhoea from birth and failure to thrive. Only one of the children developed hypoproteinaemia. Investigations showed absence of proteolytic activity in the duodenal juice. Chymotryptic and carboxypeptidase activity appeared after the addition of trypsin. The addition of a small amount of enterokinase to the juice resulted in rapid and complete activation of pancreatic proteolytic enzymes. Normal levels of trypsin, chymotrypsin and carboxypeptidase appeared in the duodenal juice showing that the precursor forms of all these enzymes were present. In the first patient enterokinase activity was assayed after gel filtration of the juice on a Sephadex G 100 column and was not detected. Enterokinase activity was similarly absent in the soluble fraction of a homogenized duodenal biopsy specimen although it was present in control mucosal biopsies from patients with coeliac disease. Duodenal biopsies from both children were normal to light and electron microscopy and had normal disaccharidase activities.

Both children had steatorrhea in early infancy but as their general nutritional state improved fat absorption became normal. Despite the absence of proteolytic activity in the duodenal juice, faecal trypsin was present in normal concentrations. In both children treatment with oral pancreatic extract resulted in prompt control of symptoms and in improved growth.

the absorption mechanisms causing sugar as well as protein intolerance

**G Meeuwisse (Lund) Renal glucosuria in glucose-galactose malabsorption**

The glucosuria of 3 fasting patients with glucose-galactose malabsorption was measured quantitatively with a sensitive method for the detection of glucose in urine. The concentration of glucose was in the range in which Clinistix gives varying results (15–40 mg/100 ml) or sometimes slightly higher. This corresponded to a moderately increased excretion of glucose per unit time which was not found in four of the parents of the patients. Two of the patients were subjected to forced diuresis by drinking large quantities of water. The glucose concentration fell to values as low as 3 mg/100 ml which was however higher than in controls with diuresis of the same magnitude. The maximal reabsorption capacity of glucose ( $Tm_0$ ) was found to be normal in 3 patients and before the attainment of  $Tm_0$  there was not much increase of the glucosuria.

The patients are probably capable of transporting glucose actively in the renal tubuli by a mechanism other than the intestinal mucosa. It was speculated that in normal individuals the mechanism responsible for the bulk transport of glucose in the small intestine has some task but only a minor one in the kidney.

**T Lindberg (Malmö) & B Karlsson (Lund) Developmental changes in intestinal dipeptidases of foetal and neonatal pig as related to changes in the ultrastructure of the mucosal cells**

The dipeptidase activities (units/mg N) on L-alanyl L-glutamic acid, L-alanyl L-proline and glycyl L-leucine are very low in porcine intestinal mucosa from early foetal development (2 cm C-R length). The activities increase uniformly up to the stage of 14 cm C-R length. The L-alanyl L-proline dipeptidase activity thereafter

remains at about the same level throughout the whole developmental period. The other two activities, however, increase considerably from the stage of 14 cm C-R length. The values registered in newborn piglets not fed with colostrum are 2–3 times higher than those from slaughtered pigs. However, these activities decrease abruptly and significantly after the newborn pig has ingested colostrum.

The electron microscopic studies reveal a striking parallelism between enzyme levels registered and appearance of microvilli in the absorptive cells. Microvilli are practically lacking in fetuses of 2 cm C-R length but begin to differentiate in the 4–5 cm fetuses. With proceeding foetal development they reach step by step the appearance earlier noted in the mucosa of newborn piglets (Mansson & Karlsson, 1966).

The results obtained complete and illustrate earlier findings in man and rat (Lindberg, 1966). Correlation between structure of mucosal cells and magnitude of dipeptidase activity thus exists in rat (Lindberg & Öwman, 1966) and pig. Moreover, in man it has been shown that intestinal dipeptidase activities are significantly reduced in coeliac disease (Lindberg *et al.* 1968) where the structure of the mucosal cells, i.e. the microvilli, are known to be affected.

The decrease of the dipeptidase activities after colostrum ingestion cannot yet be satisfactorily explained. Experiments performed *in vitro* did not demonstrate any direct inhibitory effect of porcine colostrum on the activities, while other experiments *in vivo* have shown that the decrease is connected in one way or another with the ingestion of colostrum.

**B Hadorn (Berne) Intestinal enterokinase deficiency. A newly recognized disorder of protein digestion in early infancy**

Proteolytic enzymes of the pancreas are stored in the gland as inactive precursors (zymogens). They become activated only after the pancreatic secretion has reached the duodenum. The key enzyme in this activation process is entero-

lucase a proteolytic enzyme secreted by the mucosa of the small intestine. Enterokinase converts the proenzyme of trypsin (trypsinogen) into active trypsin. trypsin then activates the other pancreatic zymogens (chymotrypsinogens and procarboxypeptidases).

Thus enterokinase is the key enzyme in this process and enterokinase deficiency must result in a complete failure to activate pancreatic zymogens.

So far 2 cases of intestinal enterokinase deficiency are known (1, 2). In both patients the disease manifested as a severe disturbance of protein digestion with diarrhoea from birth, failure to thrive, anaemia and severe hypoproteinaemia.

The biochemical evidence that the primary defect of protein digestion in these patients is due to enterokinase deficiency is provided by three observations:

1. In the duodenal fluid the activities of the pro-oligo-enzymes trypsin, chymotrypsin and carboxypeptidase are undetectable or extremely low. The addition of enterokinase to the fluid *in vitro* results in a rapid appearance of normal activities of all three enzymes. From this it must be concluded that the pancreatic zymogens are secreted normally into the duodenum but cannot be activated.

2. After Gel filtration of duodenal fluid enterokinase activity can be directly measured. It is undetectable in the patients' duodenal fluid.

3. No enterokinase activity is detected in the patients' duodenal mucosa obtained by peroral biopsy.

Enterokinase deficiency is probably a congenital deficiency of one single intestinal enzyme. Evidence that it is a primary defect rather than the effect of a more generalized small bowel disease is provided by the following observation. At the age of 17 months one of the patients was re-investigated. At this time she had following a substitution therapy with pancreatic extracts containing enterokinase fully recovered from the secondary effects of protein malabsorption. A duodenal biopsy was taken which was morphologically normal and

had normal lactase activity. Enterokinase activity however was still undetectable in the mucosal homogenate.

## REFERENCES

1. Hadorn B., Tarlow M. J., Lloyd J. K. & Wolff H. *Lancet* 1: 813 1969.
2. Polonovski C., Hadorn B. & Bieri H. Unpublished observation.

M. J. Tarlow (London), B. Hadorn (Berne) & M. W. Arthurton (Bradford) *Intestinal enterokinase deficiency. Report of 2 cases*

Two patients with intestinal enterokinase deficiency have been studied: both were girls who presented with diarrhoea from birth and failure to thrive. Only one of the children developed hypoproteinaemia. Investigations showed absence of proteolytic activity in the duodenal juice; chymotryptic and carboxypeptidase activity appeared after the addition of trypsin. The addition of a small amount of enterokinase to the juice resulted in rapid and complete activation of pancreatic proteolytic enzymes; normal levels of trypsin, chymotrypsin and carboxypeptidase appeared in the duodenal juice, showing that the precursor forms of all these enzymes were present. In the first patient enterokinase activity was assayed after gel filtration of the juice on a Sephadex G 100 column and was not detected; enterokinase activity was similarly absent in the soluble fraction of a homogenised duodenal biopsy specimen although it was present in control mucosal biopsies from patients with coeliac disease. Duodenal biopsies from both children were normal to light and electron microscopy and had normal disaccharidase activities.

Both children had steatorrhoea as early as infancy but as their general nutritional state improved fat absorption became normal. Despite the absence of proteolytic activity in the duodenal juice faecal trypsin was present in normal concentrations. In both children treatment with oral pancreatic extract resulted in prompt control of symptoms and in improved growth.



The clinical and laboratory features of trypsinogen deficiency disease appear identical with those of our patients and thus diagnosis was originally made in our first case. We suggest that enterokinase should be assayed in patients with trypsinogen deficiency disease.

A simple screening test for enterokinase deficiency has been developed. The sample of duodenal juice to be tested which is deficient in tryptic activity is incubated with juice from a patient with known exocrine pancreatic insufficiency; the latter contains enterokinase but no trypsin. After incubation the mixture is assayed for trypsin; in enterokinase deficiency trypsin will appear.

C Polonovski & H Bier (Paris) *Pseudo trypsinogen deficiency due to a lack of intestinal enterokinase*

Together with Dr Allison we studied an infant presenting during the first month of life with diarrhoea, anemia and severe hypoproteinemias. The duodenal juice showed an isolated lack of trypsin activity. Similar cases have been described by Townes (1, 2) and Morris *et al* (3) and have been interpreted as trypsinogen deficiency disease. In our case some findings made us suspect that not a complete lack but rather an abnormality of trypsinogen was present. The gelatine film test for trypsin activity in stool was strongly positive. The duodenal juice appeared to have activity towards the synthetic trypsin substrate BANA (benzoylarginine naphthylamide) but did not react with other substrates like haemoglobin gelatin and II AEE (benzoylarginine ethylester). We now attempted to activate the patient's pancreatic zymogens with bovine crystallised trypsin using short activation times and without adding calcium. With this procedure chymotrypsin and procarboxypeptidases were readily activated but no additional trypsin was found suggesting that the patient's trypsinogen was not activated.

At this point of the investigation we learned of a similar case (4) in which intestinal enterokinase deficiency was found to be the cause

of this syndrome. We therefore attempted to activate the patient's duodenal juice with a duodenal juice containing enterokinase but no trypsin (we obtained this from a patient with complete pancreatic insufficiency). With this method the patient's trypsinogen was readily activated. Incubation of the patient's duodenal juice with normal duodenal mucosa also resulted in trypsinogen activation, an ideal mucosal homogenate however failed to activate. Direct measurements of enterokinase activity in duodenal juice and mucosa of the patient were performed by Hadorn (5) and confirmed enterokinase deficiency.

Although trypsinogen of animal origin can be activated by crystalline trypsin *in vitro*, human trypsinogen appears to resist the activation with exogenous crystalline trypsin. On the other hand, the activation of human trypsinogen by enterokinase is rapid. Furthermore this process is facilitated after addition of calcium and dialysis of the duodenal juice.

Since it is difficult to activate human trypsinogen with exogenous bovine trypsin (a method used by Townes & Morris in an attempt to prove trypsinogen deficiency) we would like to raise the question whether the cases described by these authors may have suffered from the duodenal enterokinase deficiency rather than from a defect of pancreatic trypsinogen. Figarella has recently described two human trypsinogens. Therefore if trypsinogen deficiency exists one would have to admit that a common precursor of these two zymogens is involved.

An additional defect which we found in our patient was deficiency of phospholipase. Phospholipase depends for its activation from trypsin. It is not unlikely that non activation of phospholipases has caused the transitory steatorrhea which is observed in enterokinase deficient infants.

#### REFERENCES

- 1 Townes P L *J Pediatr* 66: 75 1965
- 2 Townes P L Bryson M F & Müller G J *Pediatr* 71: 220 1967

- 3 Morris M D & Fisher H A *Amer J Dis Child* 114 703 1967
- 4 Hadorn B Tarkow M J Lloyd J K & Wolff O H *Lancet* i 812 1969
- 5 Hadorn B Personal communication

Ch M Anderson (Birmingham) *The value of rectal biopsy in the investigation of small intestinal disorders*

Since the introduction in the late 1950s of peroral intestinal mucosal biopsy procedures the understanding of a number of small intestinal disorders has greatly increased. This technique has enabled the examination of "living intestinal mucosa" by various means—structural histological, biochemical and by tissue culture. Its first contribution was the demonstration of the altered villous pattern in coeliac disease ("flattened mucosa"). This appearance was thought at first to be specific to that disease but further evaluations showed it to be present from other reasons than wheat gluten intolerance. A wide appraisal of the findings of many workers can now clarify the actual diagnostic value of alterations in villous structure revealed in biopsies. The occasions in which this investigation is of real clinical value were discussed.

The technique has proved of great value in the study of enzymatic activities in the mucosa particularly in relation to the digestion of sugars. For some years the technique was of research value in this field but again an appraisal of findings now clarifies the limits of its usefulness in clinical practice.

Specific structural alterations of the mucosa in certain rare disorders of small intestinal function such as intestinal lymphangiectasis and abetalipoproteinaemia can also be revealed.

The technique still has research potential in the delineation of other disorders of enzyme function particularly in regard to protein digestion and absorption and also to the immunological aspects of the small intestinal mucosa. In these areas clinical applications is still not fully clarified.

## J Rey (Paris) *Disturbances of intestinal fat transport*

Disturbances of intestinal fat transport include all the anomalies concerned with the entry of monoglycerides and free fatty acids into the cells of the intestine, resynthesis of triglycerides, the formation of chylomicrons and the discharge of fats into the intercellular spaces and their passage in the lymphatics.

Firstly a review was given of the principal steps of digestion and absorption of fats emphasizing the role of bile salts in their solubilization as micelles. So far as resynthesis of triglycerides is concerned the regulatory mechanisms inside the cells of the intestine, the mechanisms of formation of chylomicrons and the factors which influence their distribution between the lymphatic and portal venous system were discussed.

Secondly a review was given of all the pathological conditions in which a disturbance of intestinal fat transport occurs. They comprise deficiencies of bile salts and malabsorption syndromes with atrophy of the intestinal mucosa. Furthermore are included defects in chylomicron formation either secondary to a  $\beta$ -hypoproteinaemia or idiopathic, both remarkable examples of intercellular block of fat transport facilitating the understanding of certain details of normal transport mechanisms. Finally a number of the lymphatics with stasis or lipid in the lamina propria and at the base of the absorptive cells themselves belong to this group of disorders.

## R D G Milner (Kingston Jamaica) *Hormonal and metabolic interrelationships in malnutrition*

Malnourished Jamaican infants aged between 8 and 22 months and weighing 3.2 to 8.6 kg were studied. Clinically most babies had marasmus, a few had marasmic kwashiorkor. The greatly increased growth rate of such infants made them suitable subjects for a study of the hormonal changes associated with growth.

Plasma concentration of glucose, free fatty acids (FFA),  $\alpha$ -amino nitrogen, insulin and growth hormone were measured in response to either intravenous glucagon (0.1 mg/kg) or a mixture of the 10 essential amino acids given orally (0.5 g/kg). Venous blood samples were taken before and at 3, 10, 30 and 60 min after glucagon and before and at 15, 30, 60, 120 and 180 min after oral amino acids.

Each test was performed shortly after admission (sick infants) and again 6–10 weeks later when the children were clinically recovered (well infants). Fasting levels of plasma FFA and growth hormone were higher in sick infants while insulin levels were lower.

The plasma glucose response to glucagon was greater in well infants. In both groups there were similar small rise in insulin at 3 min. FFA and  $\alpha$ -amino nitrogen levels fell. Plasma growth hormones levels rose to a maximum at 10 min and subsequently fell.

The plasma amino nitrogen level rose to a peak at 30–60 min following oral amino acids. No consistent change in plasma glucose or insulin levels occurred. FFA levels fell to a nadir at 60 min and then rose above fasting levels. In both sick and well infants there was a fall in plasma growth hormone levels over the first 30–60 min to a level which was maintained for the next 2 hours.

**Conclusion.** In these malnourished infants high plasma growth hormone levels could be further raised by intravenous glucagon or depressed by oral amino acids. Plasma insulin levels were low and responded poorly to glucagon both on admission and after recovery.

R. Gitzelmann, T. Bichi, H. Biaz, J. Lindenmann & G. Semenza (Zurich). *Localization of rabbit intestinal sucrase isomaltase with ferritin antibody conjugates*

Rabbit intestinal sucrase isomaltase complex was isolated and precipitating antisera were obtained from guinea pigs. Antibody ferritin

conjugates were prepared. After the removal of the enteric surface coat by pefraction and tryptic digestion, pieces of mucosa were exposed to the conjugates. Ferritin of antibody ferritin conjugates was localized with the electron microscope. It was shown that the antibody recognized sucrase isomaltase at the surface of the undisrupted microvilli. It was concluded that the sucrase isomaltase complex in the rabbit was membrane bound, i.e. that it constituted an integral part of the outermost layer of the microvillus membrane.

A. Holzel (Manchester). *A rare type of E. coli gastroenteritis*

From the end of December 1968 until May 1969 an unusual form of infantile gastroenteritis was encountered. 80 cases during that period showed all degrees of severity but serious forms predominated and carried a high fatality rate, particularly among infants with gross congenital malformations. In spite of the maintenance of electrolyte and acid base balance absorptive capacity of the alimentary tract was completely disrupted and any attempt of oral feeding led to a relapse of vomiting and diarrhoea. Intravenous alimentation had to be continued for periods up to six weeks. In patients who recovered intolerances for mono- and disaccharides persisted for many weeks. In the babies who succumbed liver failure had become irreversible.

The aetiological agent is assumed to be *E. coli* 0114 which has previously only been found in two outbreaks. The organism was resistant to ten of the common antibiotics but sensitive to Gentamicin and Colomycin. Since the determination of the antibiotic sensitivity the administration of the two antibiotics to all patients admitted with *E. coli* 0114 infection has greatly affected the course of the disease. There were no further deaths and intravenous alimentation in the graver forms was required only for much shorter periods. Sugar malabsorption was limited to lactose.

D H Shmerling (Zurich) & M Shmer (London) *The response of the intestinal mucosa to the intraduodenal instillation of gluten in patients with coeliac disease during remission*

The response of the intestinal mucosa to the intraduodenal instillation of boiled gluten was evaluated in two children 5½ and 8½ year old with coeliac disease in remission following a gluten free diet for 3½ years. Biochemical studies, serum IgA assays as well as dissecting, light and electron microscope investigations of small intestinal mucosal biopsy specimens were performed before the instillation of gluten and 2, 48 and 96 hours after.

No clinical, biochemical or immunological changes occurred during the period of investigation. When examined with the dissecting and the light microscope the mucosa showed after the instillation only slight changes consisting mainly of oedema and increased cellular infiltration. Ultrastructural changes were evident in the mucosa only 48 and 96 hours after the instillation and consisted of thickening, widening and coarsening of the epithelial basement membrane. Similar changes of the basement membrane of small blood vessels in the lamina propria associated with endothelial proliferation also became evident. Only subsequently an increased degeneration and shedding of villous epithelial cells through their detachment from their basement membrane as well as burrowing of round cells, mainly lymphocytes

through the villous epithelial cells appeared. The lamina propria was then heavily infiltrated with lymphocytes, plasma cells and some mast cells.

These changes suggest that gluten does not exert a direct toxic effect on the villous epithelium. They are consistent with pathological changes taking place primarily in the lamina propria possibly as a result of a delayed type immunological response.

Further similar studies on other patients are in progress.

H Loeb, P Petit, M Vansiel, M L Buyle & A Piepex (Brussels) *Congenital chloride diarrhoea*

A 6-year-old boy presented with congenital chloride diarrhoea, metabolic alkalosis (pH 7.51, total CO<sub>2</sub> 25 mEq/l), hypochloremia (83 mEq/l) and hypokalaemia (3.0 mEq/l) and furthermore weight and growth retardation.

Renal function was characterized by a reduced glomerular filtration rate (inulin clearance 59 ml/min/1.73 m<sup>2</sup>), nocthenuria with unresponsiveness to ADH, aciduria and a lowered phosphorus threshold.

Histological study of the kidney showed severe glomerular sclerosis.

The physiopathological mechanisms of this disease were discussed.

## EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY

Meeting in Interlaken September 18 1969

Round table discussion *Diagnostic criteria in coeliac disease*

Panel: H A Weyers, B Lindquist, Ch M Anderson, J Rey, D H Shmerling, J A Visa, Korpi, B Hadorn, R Gruttker

The basis for the discussion was the result of the questionnaire sent out by Dr Visa.

(see below) to the members of the Society and to some other paediatricians. Among them there had been general agreement upon one of the main questions, i.e. that coeliac disease is due to gluten intolerance and that it is usually (but not necessarily) present with symptoms and signs of malabsorption. The intestinal mucosa shows constantly typical structural alterations

Plasma concentration of glucose free fatty acids (FFA)  $\alpha$  amino nitrogen insulin and growth hormone were measured in response to either intravenous glucagon (0.1 mg/kg) or a mixture of the 10 essential amino acids given orally (0.5 g/kg). Venous blood samples were taken before and at 3 10 30 and 60 min after glucagon and before and at 15 30 60 120 and 180 min after oral amino acids.

Each test was performed shortly after admission (sick infants) and again 6–10 weeks later when the children were clinically recovered (well infants). Fasting levels of plasma FFA and growth hormone were higher in sick infants while insulin levels were lower.

The plasma glucose response to glucagon was greater in well infants. In both groups there were similar small rise in insulin at 3 min FFA and  $\alpha$  amino nitrogen levels fell. Plasma growth hormones levels rose to a maximum at 10 min and subsequently fell.

The plasma amino nitrogen level rose to a peak at 30–60 min following oral amino acids. No consistent change in plasma glucose or insulin levels occurred. FFA levels fell to a nadir at 60 min and then rose above fasting levels. In both sick and well infants there was a fall in plasma growth hormone levels over the first 30–60 min to a level which was maintained for the next 2 hours.

**Conclusion.** In these malnourished infants high plasma growth hormone levels could be further raised by intravenous glucagon or depressed by oral amino acids. Plasma insulin levels were low and responded poorly to glucagon both on admission and after recovery.

R. Gitzelmann, T. Bachli, H. Binz, J. Lindenmann & G. Semenza (Zurich). *Localization of rabbit intestinal sucrase isomaltase with ferritin antibody conjugates*

Rabbit intestinal sucrase isomaltase complex was isolated and precipitating antisera were obtained from guinea pigs. Antibody ferritin

conjugates were prepared. After the removal of the enteric surface coat by prefixation and tryptic digestion pieces of mucosa were exposed to the conjugates. Ferritin of antibody ferritin conjugates was localized with the electron microscope. It was shown that the antibody recognized sucrase isomaltase at the surface of the undisturbed microvilli. It was concluded that the sucrase isomaltase complex in the rabbit was membrane bound, i.e. that it constituted an integral part of the outermost layer of the microvillus membrane.

#### A. Holzel (Manchester). *A rare type of E. coli gastroenteritis*

From the end of December 1968 until May 1969 an unusual form of infantile gastroenteritis was encountered. 80 cases during that period showed all degrees of severity but serious forms predominated and carried a high fatality rate particularly among infants with gross congenital malformations. In spite of the maintenance of electrolyte and acid base balance absorptive capacity of the alimentary tract was completely disrupted and any attempt of oral feeding led to a relapse of vomiting and diarrhoea. Intravenous alimentation had to be continued for periods up to six weeks. In patients who recovered intolerances for mono- and disaccharides persisted for many weeks. In the babies who succumbed liver failure had become irreversible.

The aetiological agent is assumed to be *E. coli* 0114 which has previously only been found in two outbreaks. The organism was resistant to ten of the common antibiotics but sensitive to Gentamycin and Colomycin. Since the determination of the antibiotic sensitivity the administration of the two antibiotics to all patients admitted with *E. coli* 0114 infection has greatly affected the course of the disease. There were no further deaths and intravenous alimentation in the graver forms was required only for much shorter periods. Sugar malabsorption was limited to lactose.

gladin or special gladin hydrolysates are neither practical nor entirely reliable for this purpose because of variations in preparation procedures.

**4. Definition of gluten free diet.** A gluten free diet must not contain wheat, rye, barley or oats (French ble seigle, orge avoine, German Weizen, Roggen, Gerste, Hafer). Of these only wheat and rye are known with certainty to be harmful to patients with coeliac disease. Barley is supposed to be harmful. Oats may well be harmless but this point needs further investigation. Buckwheat and millet as well as corn (maize) and rice are harmless. The maximum tolerable amount of original wheat pro-

tein in wheat starch preparations made for patients with coeliac disease can not be defined as no studies on this aspect have been done.

Coeliac like manifestations may be caused in young babies also by milk protein intolerance and disaccharidase deficiency. In these cases diagnostic confusion is possible when the diet contains wheat besides milk protein and disaccharides. Furthermore it is quite possible that susceptibility to gluten is induced more easily when wheat is administered to very young babies. Hence the meeting agreed that the modern trend of introducing wheat into the diet before the age of 4 months is undesirable.

G. H. Meuwisse

## APPENDIX

### AN INTERNATIONAL INQUIRY CONCERNING THE DIAGNOSTIC CRITERIA OF COELIAC DISEASE

A summary of results Edited by J. K. Vakkari (Helsinki)

At the request of the Council of European Society for Paediatric Gastroenterology (ESPGA) a questionnaire was prepared to acquire information on how the diagnosis of coeliac disease is made in various hospitals today. The opportunity was taken of asking simultaneously about the treatment of the disease. The questionnaire was sent to 50 colleagues including all the members of ESPGA working in different hospitals and in addition to some other paediatricians interested in gastroenterology. In all 33 questionnaires were returned. The respondents were as follows: Ch. Anderson (Birmingham), S. Arsicovic (Napoli), H. Berger (Linz), J. A. Black (Sheffield), D. Boda and L. Szabo (Stockholm), J. Carr (Belts), M. Davidsson (New York), K. Deitel (Geneva), E. Eggermont (Louvain), R. Grunzler (Hamburg), J. R. Hamilton (Toronto), D. C. Heper (Torrance), T. Lindberg (Malmö), B. Lundquist and G. Meuwisse (Lund), J. K. Lloyd (London), H. Loeb (Breslau), J. Meuwisse (Paris), V. Meunier (Verona), S. Nordio (Trieste), H. Nurne (Luzern), C. Polonovski (Paris), J. Ruy and J. Jos (Paris), E. Ross, B. Hadorn and J. Guzzi (Bern), O. Sari (Berna), D. H. Slomberg (Zürich), A. Stenroos (Oslo), R. R. W. Towler (Melbourne), J. A. Waller-Smith (Sydney), H. A. Weyers, J. H. van de Kamer and E. A. J. Wouda (Utrecht), L. Wrensch (Oslo).

R. Zetterstrom (Stockholm), G. Zoppi (Ferrara) and J. K. Vakkari (Helsinki).

The first part of the questionnaire related to the definition of coeliac disease. A simple model was given for definition: coeliac disease is a malabsorption syndrome due to gluten intolerance. This model was generally accepted. Some wished to add the characteristic biopsy findings to the definition. However, a great deal of confusion arose as a result of the question concerned with the permanency of gluten intolerance. Since fifteen respondents said they had evidence of the existence of a transient gluten intolerance, 13 had doubtful evidence and five had no such evidence at all. A slight majority (18) considered that the transient form if it existed should be excluded from coeliac disease. There was rather close unanimity that patients suffering from one systemic disease such as diabetes mellitus or intolerance to other foodstuffs such as cow's milk should be called coeliacs if they otherwise fulfilled the criteria.

The main question: the diagnostic criteria of coeliac disease has been divided into two parts: the basic clinical and laboratory criteria and the testing of gluten intolerance.

The answers concerning diagnostic symptoms signs and laboratory results varied a great deal. Table 1

A majority considered, therefore, an intestinal biopsy to be indispensable for the diagnosis.

At the round table the discussion was centered around four questions. Agreement upon them was desired in order to be able to compare future research findings from different European centres.

**1 Definition of coeliac disease** It was decided that the diagnosis of coeliac disease should be restricted to patients with *permanent* gluten intolerance. Although most of the panel and the audience believed that transient gluten intolerance (if it exists) is relatively rare, it was decided that more facts about such conditions were required. For the moment a patient with absent or almost absent villi (so called subtotal villous atrophy) who shows definite improvement on a gluten free diet cannot be designated as having coeliac disease before he has been proved to normalize entirely (or almost so) on dietary treatment, not only clinically but also histologically, and subsequently to relapse after the reintroduction of gluten.

**2 The basic clinical and laboratory criteria for the diagnosis of untreated coeliac disease** Most infants and small children with coeliac disease show failure to thrive, abdominal distension and frequent bulky stools, but it is clear that asymptomatic cases exist, some of whom might have their first symptoms in adult life. No laboratory test is entirely reliable, but the faecal excretion of fat is seldom within the normal range. Many centers seemed to apply rather liberal standards for this test. Van de Kamer stressed that with a 5 day stool collection and the method described by him, indicated as method B and implying fat extraction and subsequent saponification, the upper normal limit for children on a normal diet is 3 g per day. In clinical practice absorption tests, tests of nutritional status and exclusion tests for other diseases, e.g. cystic fibrosis, bacterial or parasitic infections, etc., have to be performed. The only decisive criteria, however, are the abnormal morphology of the small intestinal mucosa, its normalization on gluten withdrawal and the reaction on reintroduction of gluten.

The biopsy should be taken in the distal duodenum or proximal jejunum. Examination of the specimen under a dissecting microscope will usually show the characteristic flat mucosa in untreated coeliac disease, but for complete evaluation histological examination is necessary. Up to now no specific enzyme deficiency (dispeptidase, disaccharidases, etc.) has been found in coeliac disease.

**3 Testing gluten intolerance** Several of the panelists with extensive experience of patients with coeliac disease were inclined to believe that formal testing of gluten intolerance may be unnecessary, but in practice after years of treatment many patients must be allowed to try gluten at least for a while. If on such a trial recurrence of the typical mucosal lesion can be demonstrated, it is usually easier to convince the patients or their parents of the necessity to continue the diet throughout life.

Initially the clinically judged improvement on a gluten free diet should be supported with simple absorption tests. Most clinicians do not reintroduce gluten again during the initial treatment of the patient (provocation test). There was agreement upon the point that in order to decide whether a patient has permanent gluten intolerance, the intestinal mucosa must first become normal before the effect of gluten is studied again. In children on a strict gluten free diet normalization of the mucosa may take one or two years. Before reintroduction of gluten a new biopsy should be taken. On resumption of a normal diet recurrence of disease is usually slow or may even be silent. Before a patient is classified as having been suffering from *transient* gluten intolerance, the intestinal biopsy should still be normal two years after the reintroduction of gluten. At present none of the members of the Society has such final proof of the existence of cases with transient coeliac disease, either because suspected cases have not been biopsied at the beginning of the disease or because they have not yet been followed up for a sufficient length of time.

The testing of gluten intolerance should be done with wheat or (wheat) gluten. At present

PROCEEDINGS OF PAEDIATRIC SOCIETIES

SCANDINAVIAN ASSOCIATION OF PAEDIATRIC SURGEONS

Fifth Meeting Sept 17-19 1969 Vedback Denmark

WINKEL SMITH MEMORIAL LECTURE

Preben Plum (Copenhagen Denmark) *On medical and surgical treatment of cerebral palsy*

SYMPOSIUM ON INTERSEX

Hennig Andersen (Copenhagen Denmark) *Pediatric and endocrinological aspects*

P Fogh Andersen (Copenhagen Denmark) *Surgical aspects A survey of 20 years experience*

Surgical treatment of intersex conditions varies from rather simple operations to more complicated reconstructions and is a fascinating subject on the borderline of pediatric surgery general surgery gynecology urology and—not least—plastic surgery This fact appears clearly from a series of intersex patients operated upon during the last 20 years partly in Rigshospitalet and Queen Louise's Children's Hospital partly in Drakonessethelmsens Hospital (Deaconess Hospital) Copenhagen

Following the classification of Hennig Andersen the largest number of patients in our series belonged to the groups of male pseudohermaphroditism (complete or incomplete testicular feminisation and female pseudohermaphroditism (adrenogenital syndrome)

In testicular feminisation (Morrison syndrome) when discovered in infancy or childhood the gonads could either be transposed into the abdomen for protection awaiting the future development of the patient at puberty or they could be removed and later replace-

ment hormone therapy instituted If in addition there is a vaginal aplasia we recommend a construction of an artificial vagina after the age of puberty our method of choice has been ulnar skin grafting using a modified McIndoe technique Congenital absence or hypoplasia of the breasts in partial testicular feminisation could be corrected successfully by implantation of prostheses (Cronin or Arion) For psychological reasons we don't tell the male pseudohermaphrodites about the true character of their gonads or genetic sex Correctly treated most of these patients will be able to have a normal life as women and get happily married

In the management of female pseudohermaphroditism (adrenogenital syndrome) a close cooperation between the pediatrician and the surgeon is of greatest importance It has been our experience that in cases of enlarged penis like clitoris this should not be preserved an infant of doubtful sex belonging to this group will never develop a well functioning penis The child is better reared as a girl and a plastic operation on the clitoris performed at an early stage before the age of 3 or 4 years We prefer a subtotal amputation of the clitoris i.e. removal of the corpora cavernosa leaving the glans or part of it attached to its blood supply An accompanying hypoplasia of the vagina is preferably corrected later

True hermaphrodites with both ovarian and testicular tissue are very rare No case happened to be included in our operative series during 20 years

So-called psychic hermaphroditism trans-



Table 1 *Diagnostic symptoms signs and investigations*

Number of respondents who consider particular symptom signs or lab tests as essential for diagnosis	Symptoms signs and laboratory tests
More than 10	Onset of symptoms after introduction of cereals prolonged diarrhoea pale bulky and of firm stools failure to gain weight and height abdominal distention abnormal faecal fat abnormal xylose absorption normal sweat tests abnormal mucosa
More than 15	Prolonged diarrhoea failure to gain weight abnormal faecal fat abnormal xylose absorption normal sweat test abnormal mucosa
More than 20	Failure to gain weight abnormal faecal fat normal sweat test abnormal mucosa
More than 25	Failure to gain weight abnormal faecal fat abnormal mucosa normal sweat test
More than 26	Abnormal faecal fat abnormal mucosa
More than 27	Abnormal faecal fat

shows how many respondents considered the particular symptoms sign or laboratory tests diagnostic. According to this the most important symptom is the failure to gain weight followed by prolonged diarrhoea and failure to grow. Abdominal distension was the most important sign. The most important laboratory and other examinations are faecal fat the intestinal biopsy sweat test and the D xylose test. These results showed a pattern of symptoms signs and laboratory findings typical of coeliac disease but it was difficult to extract from the questionnaires what everyone considered to be the minimal requirements for diagnosis and thus was the original intention of the question. With two exceptions everybody listed some symptoms and everybody agreed with the exception of the same two listed some laboratory tests. All but five regarded biopsy as essential.

The separate testing of gluten intolerance was considered necessary. The commonest method applied by everybody was that of observation during the administration of gluten free diet. Of 33 who confirmed the effect of gluten exclusion by laboratory tests and 17 by biopsy. Eighteen also suggested that a provocation test should be made after initial improvement.

Table 2 *The number of intestinal biopsies and perforations reported*

Type of instrument	No of biopsies (approx)	No of perforation
Crosby & Watson	3 895	6
Rubin tube	592	1
Hydraulic instrument	350	1
Sethus tube	100	—
	4 937	8 (0.16 %)

although the questionnaire failed to elicit correct answers on exactly when such provocation should be introduced.

The suggested lengths of treatment on gluten free diet were as follows: life long by 7 until growth ceases by 6 many years by 5 / to 2 years by 11. Thirty two respondents excluded wheat from the diet 30 rye 25 oats 26 barley 9 buckwheat and 9 millet. The questionnaire also provided some details of the technique of certain laboratory investigations. The determination of faecal fat was the most widely applied absorption test and was usually performed by the classical method of van de Kamer. The collection period varied from 2 to 6 days and carmine was often used as the faecal marker in balance studies. The other tests which indicate fat absorption were not used very much the commonest of them was the microscopic examination of stool fat. Radiosensitive methods widely used for adults were not applied in paediatric hospitals. For examination of the absorption of carbohydrates D xylose absorption tests where the ones most commonly used. The glucose tolerance test was also used to a rather large extent. In 18 instances the D xylose excretion test was applied. The dosage had large variations but similar normal values were adopted. Nine applied the D xylose tolerance test.

The questionnaires also provided some data on biopsies performed (Table 2). Eight perforations were mentioned representing 1.6 per 1000. The Crosby capsule the instrument employed most widely was also the commonest cause of perforations. Very few other complications were reported bleeding prolonged pyloric spasm transitory apnoea on introduction of the tube and impingement of the outer spring of the Crosby capsule in the oesophagus.

It is important to realise that the results of this inquiry do not solve the problem of diagnostic criteria of coeliac disease and do not impart to us the final truth about the disease. However interesting information is provided on how the different paediatricians make the diagnosis today and how they treat the disease.

and tubes. The determination of sex chromatin and hormone analysis give further support to the diagnosis.

Surgical correction of the external genitalia should be started at 3 years of age when the child is not yet sexually determined and it should be completed at 4-5 years of age. For surgical treatment of the internal genitalia I consider the age of 10-11 years most suitable.

In correction of the external genitalia of a male individual straightening of the penis is performed where curvature of erection exists. The broad fibrous plate on the underside of the penis is broken by means of small incisions when satisfactory straightening has been achieved the defect is reconstructed with the aid of two flaps of penile skin. At the second session the terminal urethra construction is made with a continuous buried strip of epithelium from the meatus out to the glans as presented by Denis Browne.

When the vagina uterus tubes and streak gonads occur in a male individual an attempt is made to extirpate these. As a rule removal of the tubes and gonads does not cause any difficulties. However extirpation of the vagina and uterus involves the risk by reason of their anatomical position that adjacent organs will be injured consequently this form of intervention should not be made. Canonically enough a remaining vagina causes no trouble or infections. The series of patients from the Department of Plastic Surgery, Sahlgrenska, Sjukhuset included 7 male individuals with a vagina 2 (aged 39-45 years) 2 (aged 13-18 years) and 3 (aged 1-5 years). In all of these persons repeated quantitative cultures have shown <1 000 bacteria per ml of urine.

In general abdominal testes are defective in spermatogenesis but as a rule they produce adequate androgenic hormone for normal or nearly normal development of secondary masculine sex characteristics. Moreover there is the risk of malignant tumours developing if the testes are allowed to remain. If a testis with a deferent duct is present an attempt should be made to transpose it through the inguinal

canal outside the body cavity. When testis and tubes are present transposition is impossible.

G Fock & O Qvist (Stockholm Sweden) *Surgical correction of the hypertrophic clitoris*

A method for conservation of the clitoris in cases of adrenal virilization in girls and in other groups of female pseudobermaphrodites is discussed as an alternative to amputation of the phallus. In the operation described the enlarged clitoris is lifted up by a pubic incision and the cavernous bodies after undermining of the skin and the prepuce are subcutaneously fixed to the suprapubic muscular fascia. The glans of the clitoris will be partially covered and the skin and the redundant prepuce form the labia minores. The patients in the authors' series of patients were operated upon at varying ages preferably at the time for reconstruction of the perineum and the vaginal opening in cases with labial fusion and urogenital sinus. The results seems to be anatomically acceptable in most of the cases treated in the way mentioned above.

## FREE PAPERS

Ove K. Mylænder & Trond Kluge (Oslo Norway) *Familial occurrence of the megaduodenum syndrome with primary degeneration of duodenal muscle layers*

A report is given on the occurrence of megaduodenum in three brothers aged 17 years 9 years and 19 months. In the oldest patient, 2 laparotomies with freeing of adhesions failed to improve the condition. Following the establishment of a duodeno-jejunostomy satisfactory drainage of the stomach and duodenum was obtained. Histological examination of biopsies from the duodenal wall revealed an extensive degeneration and fragmentation of all muscle layers. The small vessels and the ganglions of the submucous and myenteric plexus showed a normal appearance. External compression of the duodenum could be ruled out as a causative mechanism and generalized

vestism and trans sexualism might in selected cases need surgical management, but never before adult age

In a few patients with Turner's syndrome we have been able to correct the pterygium coli by means of a Z-plastic procedure, and some Klinefelter patients have had an operative correction of the gynecomastia

In addition to the typical intersex patients a series of hypospadias of all degrees have been treated—altogether nearly 500 the method of choice has been Denis Browne's urethroplasty. During the last 2 years a number of chromosome analyses were made in our hypospadias patients so far without any abnormalities however not even in some severe perineal hypospadias

Gunnar Grotte, Gabriel Reuterskiöld & Öjnar Grnäs (Uppsala, Sweden) *Intersex at the Pediatric Surgical Service in Uppsala*

R. Björndal & O. Knutrud (Oslo, Norway) *Intersex*

During a 20 years period (1950–1969) a total of 43 patients with intersex have been admitted to The Children's Clinic Rikshospitalet, Oslo. The series comprises

A Adrenogenital syndrome	24
(a) Male sex role	8
(b) Female sex role	16
B Male pseudohermaphrodites	14
(a) Male sex role	7
(b) Female sex role	7
C Female pseudohermaphrodites	1
D True hermaphrodite	1
E Agonadism	3
Total	43

Ad A (a) In this group 6 patients including 2 siblings had pure male sex organs. Two patients had bisexual external genital organs and ovaries. They had been brought up as males and their female pseudohermaphroditism was

diagnosed too late (8½ and 16 years respectively) for a change in their social sex role

Ad B The decision of the social sex role has principally been based upon the predominant external genital appearance—including urethro-(vaginography) and sino urethroscopy. In 5 patients the social sex role has been changed from male to female after years of observation. They are

	Changed
1 male pseudohermaphrodite	3 years old
1 male pseudohermaphrodite	5½ years old
1 adrenogenital syndrome	6½ years old
1 true hermaphrodite	2½ years old
1 agonadism	2½ years old

In each particular case this serious decision has been based upon intimate collaboration between pediatricians, the psychologist, paediatric psychiatrist, social worker and paediatric surgeon

During the last 10 years the surgery needed has been performed by the paediatric surgeon and we feel this point to be of great value. In this connection, it may be stressed that during recent years we have practised transposition of the clitoris instead of clitoridectomy

L. Axelsson (Gothenburg, Sweden) *Surgical treatment of gonadal dysgenesis*

Disturbances in the development of the genital organs and in gonadal differentiation may result from deviation in the chromosome formation, endocrine disturbances or from a combined form of these viz genetic defects in the enzyme system with ensuing failure of the cells in the rudimentary genital organs to the hormone normally produced

A correct diagnosis should be established during the neonatal period, when the sex of an individual is also determined. Chromosome analysis is very valuable. Nevertheless I consider that exploratory laparotomy with biopsy from the gonads is traumatizing and should be avoided and replaced by well-conducted urethrocytography which provides reliable information on the presence of the

diaper) are now being tried out in the ward. We have applied the technique in about 30 cases with different types of stomas. The result can be considered good and encouraging. In most patients skin irritation, soreness, leakage and odour disappeared.

We find the method a considerable practical advance which is in many cases preferable to the usual collecting bag system. Contact has been made with a manufacturer who plans to produce the unit commercially.

O. Justrud & H. C. Sommerichild (Oslo, Norway) *Exstrophy vesicae urinae*

Since 1960 26 patients with true exstrophy of the bladder have been treated at the Paediatric Surgery Service, Rikshospitalet, Oslo. The results of primary bladder reconstructions are extremely disappointing, as are the results from other centres according to published reports.

True continence is achieved only in some individual cases. In view of this and upon the anatomical situation of defective bladder and defective nerve supply, defective pelvis, and defective pelvic floor and defective abdominal wall, it is proposed that primary reconstruction should be carried out only in the very few cases of female patients with a large bladder.

In most cases operation should be postponed until the patient is 2 years of age. A cutaneous ectopic urethra should then be performed, or maybe rather an implantation of the ureters to the rectosigmoid. If this rectal conduit fails this may later be converted into a cutaneous conduit.

This policy will enable reduction of the number of operations which the child has to undergo. The genital area is left for better plastic repair and the risk of irreversible renal damage is minimized.

■ Fogh Andersen (Copenhagen, Denmark) *Congenital duplication and diverticulum of the male urethra*

Two boys with the rare congenital malforma-

tion, duplication urethrae, were operated on. One had an accessory meatus on the dorsum of the glans surrounded by a hemispherical prominence simulating an extra micro-glans. An urethral channel could be traced about 2 cm backwards from the accessory opening. Primary excision was very easily made and a good cosmetic result achieved.

The other case was a complete duplication from the corona of the glans through the dorsal part of the entire penis up to the bladder neck, and persistent "incontinence" and fever attacks were present. After excision of the distal major part of the accessory urethra, no incontinence persisted; the fever attacks almost disappeared and the cosmetic result was excellent. Transurethral excision or ligation of the proximal part might have been better, however, leaving the distal accessory urethra open.

A case of large diverticulum formation of the major part of the penile urethra associated with chronic urethritis in a boy aged 2 years was treated in two stages: first splitting up the ventral wall of the urethra and excision of the superfluous mucosa, thus producing an artificial hypospadias. In a second stage of Denis Browne urethroplasty was performed with a satisfactory cosmetic and functional result.

Jaakko Elo (Helsinki, Finland) *Refluxing ureters in children*

Since September 1965 150 children with pyelonephritis have been examined at the out-patient urological clinic of Aurora Hospital. Of these patients 124 were girls and 26 boys.

Vesico-ureteral reflux was found in 53 girls and 12 boys. This gives an incidence of 43% for reflux in this series. The reflux material also includes four girls without any urinary infection who attended for the treatment of enuresis. The total series consists of 69 children with vesico-ureteral reflux. Of these 28 children were treated operatively and 41 conservatively.

Twenty-six patients were operated upon by application of the Poincaré-Leadbetter tunnel

neuromuscular disease could also be excluded

It is suggested that this type of megaduodenum is due to a primary muscular degeneration and dysfunction which has previously not been reported. It seems to follow a hereditary pattern and may be connected with similar dysfunctions of the bladder and large intestine.

Three patients, all with intrinsic obstruction

B. Brook Jacobson & C. M. Madsen (Odense, Denmark). *Neonatal duodenal obstruction*

During the years 1957–1968, 20 cases of neonatal duodenal obstruction were treated at Odense County and City Hospital, surgical department K and pediatric department J.

Ten patients had extrinsic obstruction (6 boys and 4 girls) and ten intrinsic obstruction (6 boys and 4 girls). Five patients with intrinsic obstruction had severe associated anomalies. Two had Down's syndrome.

In all cases the extrinsic obstruction was caused by malrotation with duodenal compression in 8 cases associated with volvulus of the small intestine. They were all treated with Ladd's operation. In no case was intestinal resection necessary.

Of the cases with intrinsic obstruction, an ulnar pancreas was found at operation in 7 cases. 2 cases had duodenal stenosis and 1 case diaphragmatic obstruction. In 5 cases duodeno-jejunostomy was performed and in 4 cases duodeno-duodenostomy. On the case with diaphragmatic obstruction a plastic operation was performed.

Three patients, all with intrinsic obstruction, died postoperatively. One with a birthweight of 1,500 g died three days after the operation. The two patients with Down's syndrome died 5 1/2 and 8 months after the operation.

Two patients treated by duodeno-duodenostomy were successfully operated upon for intestinal obstruction caused by adhesions 5 and 7 months after the primary operation.

Fourteen patients have been followed up from 3 to 11 years after the operation. None of these has had gastrointestinal disturbances.

A. G. Coran, R. Bjørdal, S. Eek & O. Knutrud (Oslo, Norway). *The surgical management of small intestinal aganglionosis*

Six cases of aganglionosis of the entire colon and the terminal ileum were reported.

To be published in *Surgery*, Oct. 1969.

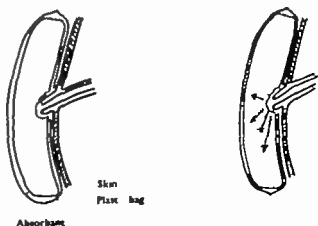
N. Øhre Nilsen (Copenhagen, Denmark). *Abdominal wall defects experimentally produced in chicken embryos*

R. Bjørdal (Oslo, Norway). *A new method for the collection of urine in urostomies*

In children with some kind of urinary diversion the method most commonly applied is that with the collecting bag.

We all know the great practical problems mainly arising from leakage and skin irritation with this equipment. At the Rikshospitalet Pediatric Surgical Service, Oslo, a new principle, invented by the mother of a myelomeningocele patient, has been employed during the last 6 months. This new technique, entitled Uro-sorb, has the following characteristics: A water-absorbing paper diaper is placed in a thin plastic bag with a suitable opening for the stoma. Ureterostoma (ileostoma) is put into the opening and the plastic bag is then placed on the abdomen with slight pressure. The urine will now drip into the absorbing material and be absorbed—the principle being the Wick effect (see figure).

Various fixation belt arrangements for application of the absorbing unit (plastic bag +



B L Lindström O Lindfors M Westerlund  
& B Kuhlbeck (Helsinki Finland) *Kidney  
transplantation in children—a follow up*

In September 1968 at the annual meeting of  
The Scandinavian Association of Paediatric  
Surgeons presentation was made of a series of  
4 children who had received kidney trans-  
plants (*Acta Paediat Scand* 58 422 1969)  
One of the children died in postoperative  
bleedings from gastrointestinal ulcers with the  
transplant still completely normal What hap-  
pened to the three other children during the  
succeeding years?

*Case 1* Girls age 11 at time of transplan-  
tion follow up 31 months Increase in height  
3 cm and weight 24 kg Mammæ and pubic  
hair in accordance with age No menarche

*Case 2* Girl age 11 at time of transplan-  
tion follow up 16 months Increase in height  
2 cm and weight 14 kg Mammæ and pubic  
hair in accordance with age No menarche

*Case 3* Boy age 13 at time of transplan-  
tion follow up 16 months Increase in height  
7 cm and weight 4 kg No Cushing symptoms

All 3 patients are in good condition blood  
pressure is normal and retinal changes have  
regressed totally No osteoporosis has been ob-  
served All the children attend school move  
actively and are no longer bound to dialysis  
centres The routine examinations are made at  
local hospitals

Erin Struve-Christensen & Jørgen Lejerstoft  
Jensen (Copenhagen Denmark) *Winckel-  
mann's operation in boys*

Since 1898 the name of Winckelmann has been  
connected with partial excision and aversion  
of the hydrocele sac

In Gentofte Hospital—Copenhagen—76  
boys under 15 years of age were treated for  
congenital hydrocele of the testis or funicul in  
the period 1960 to 1967

Sixty six of the hydroceles were located in  
the testis 42 on the right side 18 on the left  
side and 5 were bilateral

Ten funicular hydroceles were evenly distri-  
buted with 5 on either side

A follow up of 72 patients has been effected  
after a postoperative period of from 1 to 7  
years

Twenty seven of the patients were operated  
on by application of Winckelmann's principle  
and 11 of these developed recurrence of the  
hydrocele mostly within the first years They  
were all subjected to re-operation with ligation  
of the patent processus vaginalis without fur-  
ther recurrence

Primary ligation of the patent processus va-  
ginalis was performed in another 27 patients  
one of these had a recurrence

Thirteen patients were treated with both li-  
gation of the patent processus vaginalis and  
Winckelmann's operation No recurrence was  
observable

A funicular hydrocele was excised in 6 pa-  
tients and other methods were applied in an  
other 3 cases

Consequently Winckelmann's operation can  
not be recommended in child surgery

We now perform ligation of the patent pro-  
cessus vaginalis in the way described by Mc  
Kay Fowler & Barnett in 1959

C M Madsen & H W Vennits (Odense Den-  
mark) *Traumatic haemobilia*

A report is made on a case of traumatic hae-  
mobilia in a boy aged 5 years After moderate  
trauma in the region of the liver the primary  
symptoms disappeared rapidly A week later  
pain resembling hepatic colic occurred accom-  
panied by melaena The diagnosis was estab-  
lished from this typical clinical picture and  
confirmed by arteriography The cavity in the  
liver was opened at operation, and the dam-  
aged vessels and biliary passages were sutured  
The subsequent course was uncomplicated

A review of the literature has led to the  
conclusion that arteriography is an important  
pre-operative investigation and that operation  
should consist either of opening the cavity with  
suture of vessels and biliary passages or par-

lin technique The operation was performed on 40 ureters the reflux had disappeared in 31 ureters The results in respect of 8 operated ureters have not yet been checked Heminephroureterectomy was performed in 2 cases and cutaneous ureterostomy in only 1 case

The original method of Politano-Leadbetter has proved to be safe and reliable of my patients subjected to operation by this technique two developed a postoperative stricture These two were reoperated upon and the stricture has disappeared In one of these the reflux recurred unilaterally after the reoperation

Of the 20 patients successfully operated upon chemotherapy was discontinued in 15 these children are progressing well without any drugs In 5 cases, a recurrence of infection necessitated the reinstitution of chemotherapy

The reflux disappeared in 12 patients treated conservatively of whom 7 are getting well without chemotherapy Chemotherapy is still being administered to the remaining 5 to ensure a good result or to treat recurrences of infection

Niels Kroghgaard (Copenhagen Denmark)

*Repeated urinary tract infection and dysfunction of the lower urinary tract in infancy and childhood*

A new physiological method is presented including micturition cinematography combined with simultaneous, suprapubic intravesical pressure—flow recording This method makes it practicable to evaluate the lower urinary tract in infancy and childhood with great accuracy The values of intravesical pressure and urine flow are given, along with a description of the syndrome, urethral dysfunction in female children Bladder neck obstruction has not been demonstrated in female children moreover it has not been possible to prove genuine lower urinary tract obstruction in them Lower urinary tract obstruction in male children is demonstrated as valvulae urethrae congenitas and strictura urethrae Bladder neck obstruction in male children is an ex-

tremely rare disease, and is consequently without numerical importance for the relatively large group of patients who present trabeculation reflux and repeated urinary tract infection Longstanding infection did not give rise to bladder neck obstruction Bladder outlet obstruction in childhood is the cause of only a small part of the repeated urinary tract infections

Jan Gierup (Stockholm, Sweden) *The urinary flow in normal infants and children*

The urinary flow was studied in 216 boys and 180 girls, aged one day to 16 years, by the use of the method described previously in which the urinary flow is converted into air flow which is recorded via pneumotachograph—differential pressure manometer

Considerable variations were observed in the micturition pattern of different individuals With increased micturition volume the flow curve displayed a plateau formation in the age group 4–13 years this plateau seems to be typical of children It occurred with decreasing incidence in older children

The maximum flow was positively correlated to the volume of voided urine as in adults In girls no differences were demonstrable between age groups In boys of 0–14 years, the increase in maximum flow with volume was more pronounced than in boys aged 4–16 years In the age group 4–16 years, the increase in maximum flow with volume in girls showed a more pronounced increase than in boys

An attempt was made to correlate the maximum flow with height, weight body surface and age respectively The correlation coefficients were small and about the same for all the parameters Thus the maximum flow was correlated mainly with the micturition volume

Growth seems to exercise little influence upon normal emptying of the bladder Micturition dynamics in normal infants and children seem to be determined principally by physiological and hydrodynamic factors

B. L. Lindström O. Lindfors M. Westerlund  
& B. Kuhlbeck (Helsinki Finland) *Kidney  
transplantation in children—a follow up*

In September 1968 at the annual meeting of The Scandinavian Association of Paediatric Surgeons presentation was made of a series of 4 children who had received kidney transplants (*Acta Paediatr Scand* 58 422 1969). One of the children died in postoperative bleedings from gastrointestinal ulcers with the transplant still completely normal. What happened to the three other children during the succeeding years?

*Case 1* Girl age 11 at time of transplantation follow up 31 months. Increase in height 3 cm and weight 24 kg. Mammian and pubic hair in accordance with age. No menarche.

*Case 2* Girl age 11 at time of transplantation follow up 16 months. Increase in height 2 cm and weight 14 kg. Mammian and pubic hair in accordance with age. No menarche.

*Case 3* Boy age 13 at time of transplantation follow up 16 months. Increase in height 7 cm and weight 4 kg. No Cushing symptoms.

All 3 patients are in good condition, blood pressure is normal and renal changes have regressed totally. No osteoporosis has been observed. All the children attend school, move actively and are no longer bound to dialysis centres. The routine examinations are made at local hospitals.

Eivm Strøm Christensen & Jørgen Lejreth  
Jensen (Copenhagen Denmark) *Winkel  
mann's operation in boys*

Since 1898 the name of Winkelmann has been connected with partial excision and aversion of the hydrocele sac.

In Gentofte Hospital—Copenhagen—76 boys under 15 years of age were treated for congenital hydrocele of the testis or funicul in the period 1960 to 1967.

Sixty six of the hydroceles were located in the testis, 42 on the right side, 18 on the left side and 6 were bilateral.

Ten funicular hydroceles were evenly distributed with 5 on either side.

A follow up of 72 patients has been effected after a postoperative period of from 1 to 7 years.

Twenty seven of the patients were operated on by application of Winkelmann's principle and 11 of these developed recurrence of the hydrocele, mostly within the first years. They were all subjected to re-operation with ligation of the patent processus vaginalis without further recurrence.

Primary ligation of the patent processus vaginalis was performed in another 27 patients, one of these had a recurrence.

Thirteen patients were treated with both ligation of the patent processus vaginalis and Winkelmann's operation. No recurrence was observable.

A funicular hydrocele was excised in 6 patients and other methods were applied in another 3 cases.

Consequently Winkelmann's operation can not be recommended in child surgery.

We now perform ligation of the patent processus vaginalis in the way described by McKay, Fowler & Barrett in 1959.

C. M. Madsen & H. W. Vennits (Odense Denmark) *Traumatic haemobilia*

A report is made on a case of traumatic haemobilia in a boy aged 5 years. After moderate trauma in the region of the liver the primary symptoms disappeared rapidly. A week later pain resembling hepatic colic occurred accompanied by melæna. The diagnosis was established from this typical clinical picture and confirmed by arteriography. The cavity in the liver was opened at operation and the damaged vessels and biliary passages were sutured. The subsequent course was uncomplicated.

A review of the literature has led to the conclusion that arteriography is an important pre-operative investigation and that operation should consist either of opening the cavity with suture of vessels and biliary passages or par-



total resection of the liver with removal of the lesion. If no other treatment is possible, ligation of the hepatic artery can be performed.

H C Sommerschild, T Kluge & A Flatmark (Oslo, Norway). *Sinusoidal obstruction as a cause of portal hypertension in children and young adults*

Two patients with portal hypertension were operated upon at 7 and 21 years of age respectively. The portal vein was patent in both cases and the liver appeared normal on gross examination.

Light and electron microscopic studies of wedge liver biopsies revealed a marked hypertrophy of Kupffer cells and narrowing of the sinusoidal lumina. There was a heavy perisinusoidal fibrosis with obliteration of the space of Disse. The hepatic parenchymal cells and the portal tracts were normal.

Pre- and post-sinusoidal obstructions could be excluded in both patients. The observations suggest that in the present the portal hypertension is induced by a flow obstruction at the sinusoidal level. These findings seem to add a new aspect to the pathogenesis of the "idiopathic" portal hypertension. (To be published in *Surgery*.)

Hans Chr Børresen, Arnold G Coran & O Knutrud (Oslo, Norway). *Parenteral feeding of newborns undergoing major surgery*

The ultimate success of operations on newborns often depends upon complete parenteral feeding for extended periods of time. A normal newborn requires about 100 cal/kg body weight in 24 hours, which must be supplied in a fluid volume of about 140 ml. In human milk about 50% of the calories are provided by fat. Since this has not been possible in parenteral feeding hitherto, strongly hypertonic solutions of carbohydrate have been used to cover the infant's high caloric need. This hypertonicity necessitates the use of the superior or inferior vena cava for infusions. Al-

though peripheral phlebitis is thus avoided, the tendency to glucosuria and osmotic diuresis persists.

The present communication describes an improved parenteral feeding programme in terms of amounts given/kg body weight in 24 hours. The corresponding figures for breast feeding are stated below in parenthesis. The metabolic performance of the feeding programme has been analysed by multiple balance studies.

Fat intake is increased to 4 g (6 g) administered as 20% Intralipid® Vitrum. The proper clearance of this fat from the serum is ensured by the addition of 300 IU of heparin/kg body weight to the amino acid solution. This small amount of heparin was shown to normalize the otherwise increased triglyceride level.

A quantity of 3 g of amino acids (18 g) is given as a commercial solution of crystalline amino acids, viz Aminofusin L® forte Pfriemmer. The Aminofusin is mixed with 10% invertose, the total amount of carbohydrate being 12 g (10.5 g)/kg. Both a multivitamin preparation and electrolytes are added to this mixture. The total content of K<sup>+</sup>/kg body weight is 1.75–2.75 mEq (1.8 mEq) of Ca<sup>++</sup> 1 mEq (1 mEq absorbed from mother's milk) and of phosphate 1.15–2.20 mmoles (0.6 mmoles absorbed from mother's milk). The Aminofusin provides 0.15 mEq Mg<sup>+</sup> (0.17 mEq absorbed from mother's milk).

Balance studies in four patients for periods of 3 days to 4 weeks show that the feeding programme sustains positive nitrogen balances even in the immediate postoperative period of the same order of magnitude as in normal infants in the second week of life, i.e. about 200 mg N retained/kg 24 hours. The retention of K<sup>+</sup> was close to 4 mEq/kg N. This is compatible with the synthesis of normal soft tissues, the K<sup>+</sup>/N element ratio of which is between 3 and 4 mEq/g. The supply of Mg<sup>+</sup> was however probably somewhat too low, since the Mg<sup>+</sup>/N retention ratios were considerably lower than the Mg<sup>++</sup>/N element ratio of soft tissues, which is usually close to

0.6 mEq/g N Breast feeding sustains a  $Mg^{+}/N$  retention ratio close to 0.6 mEq/g which would probably not be sufficient if the deposition of  $Mg$  in the skeleton were not limited by a short supply of phosphorus in mother's milk. The  $P/N$  retention ratio varied between 54 and 207 mg  $P/g$  N. The  $P/N$  retention ratio during breast feeding is close to 80  $mEq/g$   $P/g$  N while bottle feeding increases the ratio to between 170 and 250. The  $P/N$  element ratios of soft tissues are usually between 60 and 85 mg  $P/g$  N. About 90% of the calcium was retained as would be expected in view of the relatively liberal supply of phosphorus. The retention of calcium confirms our view that infants as opposed to adults confined to bed require Ca, Mg and phosphorus to cover the needs of their growing skeleton.

A practical advantage of the feeding programmes outlined above is that small veins tolerate the infusions for between 2 and 4 days. This results from the reduction in the hypertonicity achieved through the liberal use of fat and from the simultaneous infusion of the fat emulsion and the mixture of amino acids etc. Adjustment of the pH of the latter solution might further improve the tolerance.

Jørgen Bech Hansen & Svend Arne Pedersen  
(Odense, Denmark) *Intussusception in infancy and childhood*

During the period 1936-65 196 cases of intussusception in children were treated. The duration of the symptoms in 63.3% of the cases was less than 24 hours and primary barium enema was used in 173 cases. 5 patients were operated upon primarily under the diagnosis intussusception and 18 patients were operated upon for other conditions. A second operation was necessary in 68 of the 173 cases. In 10 cases the intussusception was induced by organic lesions in the bowel all of these being of a benign nature. A follow up examination showed that the rate of recurrence calculated as a decrement series was 67.8%.

There is no statistically significant difference in the recurrence percentage following barium enema and that following primary and secondary operation. The fatality of the total material was 3.6%. After 1949 95 cases were treated without fatalities.

Primary operative treatment was applied in only a few cases in which the patient was dehydrated and the abdomen distended or with peritoneal reaction. In the remaining cases the intussusception was primarily treated by barium enema.

Th. Elhénpreis, J. Gierup & R. Lagercrantz  
(Stockholm, Sweden) *Treatment of regional enterocolitis in children and adolescents*

A series of 35 patients with regional enterocolitis is presented. Of these 31 were operated upon. 27 had colonic localization of the disease. 24 of them with the small intestine engaged as well. Medical therapy had no more than a temporary effect and most of the patients. 23 were subjected to surgical therapy by reason of chronic progressive disease with grave effects upon weight growth and the development of puberty.

The recurrence rate was high—about 50%. Wide spread disease seemed to have an unfavourable prognosis. Resections in the distal part of the colon with anastomosis is not recommended in cases with perianal and perirectal manifestations. The importance of careful preoperative assessment of the extent of the lesions and of a radical excision is stressed. Notwithstanding the high recurrence rate surgery is our best choice of treatment in the majority of patients at present.

Johannes E. Bock (Odense, Denmark) *Ano-rectal atresia with rectocoloanal fistula*

Rectal atresia with the persistence of cloaca account for about 3% of anorectal malformations. The anomaly is a "high atresia" and is attributable to disturbance in the mechanism of the division of the embryonic cloaca.

A female child with high rectal atresia, especially the pull through operation with double vagina with two separate uteri and a sacro abdomino perineal approach. The prognosis for fecal and urinary continence is discussed.

There were no associated anomalies except for a persistent omphalo mesenteric duct. The therapeutic procedure is demonstrated.

*G R Wallgren*

*Aurora Hospital, Helsinki, Finland*

NEW BOOKS RECEIVED

- **Kiv (ed)** *Nutrition in preschool and school age*  
Symposia of the Swedish Nutrition Foundation VII  
154 pp Almqvist & Wiksell Stockholm 1970  
Sw kr 45.-
- E **J Brewer Jr (ed)** *Juvenil rheumatoid arthritis*  
Major Problems in Clinical Pediatrics vol VI 231  
pp illus W B Saunders Philadelphia London and  
Toronto 1970 \$5.65
- **Henningberg (ed)** *Neuromuscularphysiologie* Abhand-  
lung aus dem Bundesgesundheitsamt Heft 8 91 pp  
Springer Verlag Berlin Heidelberg and New York  
1969 DM 22 US \$6.10
- V **A. Cowie** *A study of the early development of*  
*monkeys* 110 pp Pergamon Press Oxford 1970  
US \$8.00
- E **Gourdeau (ed)** *The hemophilic and his world*  
Bibliotheca Haematologica no 34 214 pp illus  
Proc 5th Congr World Fed of Hemophilia Mon-  
tréal 1968 Karger Basel and New York 1969  
DM 56 US \$13.45
- **Oetiker (ed)** *Neurologie im Kindesalter I*  
*Pediatrische Fortbildungskurse für die Praxis* vol  
27 117 pp illus Karger Basel and New York  
1970 DM 30 US \$7.20
- A **Prill** *Die neurologische Symptomatologie der akuten*  
*und chronischen Nerveninfektionen* 177 pp  
illus Springer Verlag Berlin Heidelberg and New  
York 1969 DM 64.-
- P **F. Bray** *Neurology in pediatrics* 514 pp illus  
Year Book Medical Publ Inc., Chicago Ill. 1969  
US \$23.50
- **N. Barnard & V. Scheure** *Die Chirurgie der*  
*häufigsten angeborenen Herzmussbildungen* 152 pp  
illus Springer Verlag Berlin Heidelberg and New  
York 1969 DM 12.80 US \$3.20
- F **Unterharnscheidt D. Jachnik & H. Gott.** *Der Fall*  
*Leucomangel* Monographien aus dem Gesamtgebiete  
der Neurologie und Psychiatrie Heft 128 232 pp  
illus Springer Verlag Berlin Heidelberg and New  
York 1969 DM 68.-
- R **Delavayre** *Le problème de l'hérédité dans les*  
*syndromes épileptiques* 236 pp Expansion Scienti-  
fique Française Paris 1969 43 F
- E **Goth** *Pathophysiologie und Klinik der Fettsucht*  
140 pp Akademie Kiado Budapest 1969 US  
\$6.00
- C **C. Mabry I E. Roedel R E. Gervodan & J. A.**  
**Koeplke** *Recent advances in pediatric clinical patho-*  
*logy* 247 pp illus Grune & Stratton New York  
and London 1969 Price not given
- X **XII congrès de l'Association des Pédiatres de**  
**langue française** Strasbourg 1969 Vol 1 *Alimentation*  
*du prématuré* Vol 2 *Réanimation du nouveau*  
*né* Vol 3 *Macropolymécharonoses et splé-*  
*nogénioloses* Expansion Scientifique Française  
Paris 1969 Price not given
- E **Davis** *Rheumatic fever Clinical ecological and*  
*forensic aspects* 160 pp Thomas Springfield Ill  
1969 US \$9.75
- M **Stefanini (ed)** *Progress in clinical pathology* vol  
II 385 pp illus Grune & Stratton New York  
and London 1969 US \$19.50
- I **Schulman (ed)** *Advances in pediatrics* vol 18  
446 pp Year Book Medical Publ Inc Chicago  
Ill 1969 US \$15.00

## BOOK REVIEWS

N S Scrimshaw C E Taylor & J E Gordon *Interactions of nutrition and infection* World Health Organization Geneva 1968 329 pp £2 14s US \$9.00 sFr 27.00

Only few physicians will be surprised to learn that kwashiorkor is rarely due only to malnutrition infection usually of the intestinal tract plays an important additional role On the other hand the fatality rate of infectious disease is greatly influenced by reduced host resistance due to nutritional deficiencies The book barely elucidates the mechanisms behind these well known facts as far as hitherto investigated It is a comprehensive review and a synthesis of the available literature on the clinical epidemiological and experimental aspects concerning the two kinds of interaction involved Effect of infection on nutritional state and effect of nutrition on infection The interaction may be synergistic antagonistic or absent

The negative effect of infection on nutrition is mainly encountered as the result of acute diarrhoea or of chronic infestation by parasites especially those invading the gut For separately grouped nutritional deficiencies the effect of infection by different micro organisms and helminths have been systematically listed Malnutrition is usually synergistic with infectious disease When virus protozoa or helminths are involved however antagonism sometimes does occur which seems to be very rarely the case in bacterial or rickettsial infections Fungal infections have hardly been mentioned because of the scarce information available A particular chapter deals with the weaning diarrhoea a good example of the complex nature of the interactions described in other parts of the book

The book is of major importance for physicians and other scientists engaged in the efforts of improving public health in the large regions of the world where malnutrition is frequently recognized Even for clinicians working in more prosperous areas valuable information can be extracted For ten years the authors have jointly been collecting and evaluating the literature in this field Their final choice of nearly 1500 references tells something about the amount of reading behind this No 57 of the World Health Organization monograph series

Gunnar Meeuwisse

F Vasella (ed) *Aspekte der pädiatrischen Neurologie* Pädiatrische Fortbildungskurse für die Praxis No 24 113 pp S Karger Basel New York 1968 sFr/DM 26—

This volume is one in a series written for postgraduate courses in pediatrics It contains good rather

short reviews of some of the actual problems in pediatric neurology F Vasella Bern has written a very instructive and informative chapter on neurologic examination of infants with tables of motor and mental development The surveys of cerebral tumours in childhood by E Zander Lausanne and of hydrocephalus by M Bettex Bern are easy to read relatively short but still with all the important aspects on the problems Other subjects which are reviewed are retinal changes in degenerative disorders of the nervous system acute hemiplegias in childhood leucodystrophies and head injuries Most of the chapters are followed by a short list of up to date references but some have very few or none at all which is a pity for the interested postgraduate reader

Ingrid Bjørre

B Jelliffe *Infant Nutrition in the Subtropics and Tropics* Second ed 335 pp illus World Health Organization Geneva 1968 £2 14s

This monograph from WHO was first published 1955 and during the following 13 years there has been a slowly increasing awareness among people in the developed countries of the world of the need for them to participate in the struggle against malnutrition and diseases in the underdeveloped countries For those who are interested in understanding more about these problems this monograph gives a good introduction After a short historical survey about infant feeding since ancient civilization there is a presentation of infant feeding practices in about 50 less developed countries all over the third world Some common patterns of infant feeding are seen in most of the countries e.g. an increased frequency of the failure of lactation among mothers in the lower socio economic group living in urban areas often associated with unsuccessful attempts at bottle feeding Animal milk seems to play very little part in the feeding of the majority of poorer infants Eggs and other protein food are also inadequately used Carbohydrate in some form is often the sole dominating foodstuff for infants As a result kwashiorkor and nutritional marasmus often occur and the chapter of the monograph entitled present status of nutritional disease among young children in the subtropics and tropics deals mostly with these two protein calorie malnutrition syndromes The author gives a short concentrated survey of the syndromes with special emphasis on the importance of early diagnosis and understanding of the miscellaneous factors in their etiology He also gives recommendations of treatment suitable for large hospitals as well as for rural hospitals In the chapters dealing with methods

improving infant feeding in the subtropics and tropics and prevention of nutritional diseases the author stresses the importance of clear-cut planning and the subsequent evaluation of the effectiveness and cost of the suggested programmes. This is also a question of selection of programme priorities and is different from country to country. The importance of not forgetting maternal nutrition is mentioned. As a general recommendation for infant feeding the monograph repeatedly stresses the use of breast feeding alone for six months; this is in fact regarded as the only way to survive in several parts of the world. From 6 months onwards mixed feeding is introduced with semisolid food that is locally available and acceptable and should be rich in vegetable and if possible animal protein given together with breastmilk thus saving the child from kwashiorkor when passing from the first six 6 months of life to the age of 2-3 when it can have full adult diet. To be able to change the pattern of infant feeding it is important to have nutritional education. The last two chapters of the book are dealing with this problem. To be able to overcome the difficulties a nutritional educator must know a lot about factors determining people's behaviour: have good knowledge about local customs, traditions and beliefs. The author discusses all these problems in some detail and analyses the obstacles to success. Among others is the dangerous influence of commercial advertising of milk food on mothers who have neither money nor education nor the kitchen facilities to handle such products. If the fall for the temptation of being modern their child will often get an overdone contaminated mixture low in nutrients and high in bacteria. At the end of the book there are 15 appendices with short very practical advice on several topics dealt with in the text before. There are also 26 illustrative photographs and about 700 references of which perhaps too many are old. This book of a very experienced author gives a lot of facts and information about the life that is a reality for the majority of people in this world.

Bo Reidel

D H Scherzberg, H Berger & A Prader (eds) *Symposium on Intestinal Absorption and Malabsorption*. 15 pp. illus. S. Karger, Basel, New York, 1968. Dfl 50.— US \$12.00.

The proceedings of a symposium on intestinal absorption and malabsorption at Zurich 1967 in honour of Prof Hocher are published in this book. As the contributors are well-known biochemists, paediatricians and gastroenterologists the contents are of good quality. The book demonstrates clearly that the amount of information of normal and abnormal conditions in the gastrointestinal tract is rapidly increasing.

The first part of the book deals with physiological problems such as the digestion and absorption of sugars and fats. Margot Shmer presents beautiful elec-

tron micrographs in a chapter on the dynamic morphology of normal and abnormal small intestinal mucosa. In spite of the fact that Prof Prader remarks in a welcome address that the programme of the symposium is more restricted than the title indicates (e.g. coeliac disease and cystic fibrosis is not included) it is nevertheless surprising that nothing is said about the digestion and absorption of proteins under physiological conditions. This drawback of the book is however to some extent neutralized by two papers on amino acid transport in the second part of the book dealing with pathological conditions. S. Segal contributes with a paper on tissue transport defects of dibasic amino acids and Ch. R. Scriver on factors influencing uptake of amino acids in kidney and intestine. Other papers in this part deal with methionine malabsorption, mono- and disaccharide malabsorption, chloride malabsorption and selective malabsorption of vitamin B<sub>12</sub>.

The last part of the book consists of three valuable chapters on general clinical problems: normal and defective production of immunoglobulins in the intestinal tract by P. A. Crabbe and J. F. Heremans; malabsorption and steatorrhea by A. F. Read and effects of extensive intestinal resections in childhood by A. W. Williamson.

Each paper is completed by a reference list and a discussion. This book is of value for physicians not only paediatricians interested in the gastrointestinal field of medicine.

Tor Lundberg

H. M. van Praag (ed) *Brain damage by inborn errors of metabolism*. De Erven F. Boek N. V. Harlem, 1968. 126 pp. US \$3.75.

This society works in an interdisciplinary setting to work with the problems concerning the relations between cerebral function and behavior—or disturbed function and disturbed behavior. The present publication consists of a number of papers given at a symposium, held in Amsterdam 1967 on the subject of inborn metabolic disorders giving rise to brain lesions.

The inborn errors of amino-acid metabolism are discussed in several chapters. A list of the most important disorders known to day is given. Aspects of pathogenesis, diagnosis, treatment and control are considered. The subheadings are not treated separately however and there is some overlap of subject matter in different papers. Nevertheless there is much valuable information in this part of the book.

Another paper by W. H. H. Tegeler gives a qualified and detailed picture of the inborn errors of metabolism of the thyroid gland including biochemical, histological and clinical features.

According to the intentions of the society there is a survey of the intellectual/psychological complications of brain damage by these disorders and a brief outline of the neurological examination of the infant is also presented.

## BOOK REVIEWS

N S Scrimshaw C E Taylor & J E Gordon *Interactions of nutrition and infection* World Health Organization Geneva 1968 329 pp £2 14s US \$9 00 sFr 27 00

Only few physicians will be surprised to learn that kwashiorkor is rarely due only to malnutrition in infection usually of the intestinal tract plays an important additional role. On the other hand the fatality rate of infectious disease is greatly influenced by reduced host resistance due to nutritional deficiencies. The book barely elucidates the mechanisms behind these well known facts as far as hitherto investigated. It is a comprehensive review and a synthesis of the available literature on the clinical epidemiological and experimental aspects concerning the two kinds of interaction involved. Effect of infection on nutritional state and effect of nutrition on infection. The interaction may be synergistic antagonistic or absent.

The negative effect of infection on nutrition is mainly encountered as the result of acute diarrhoea or of chronic infestation by parasites especially those invading the gut. For separately grouped nutritional deficiencies the effect of infection by different microorganisms and helminths have been systematically listed. Malnutrition is usually synergistic with infectious disease. When virus protozoa or helminths are involved however antagonism sometimes does occur which seems to be very rarely the case in bacterial or rickettsial infections. Fungal infections have hardly been mentioned because of the scarce information available. A particular chapter deals with the weaning diarrhoea a good example of the complex nature of the interactions described in other parts of the book.

The book is of major importance for physicians and other scientists engaged in the efforts of improving public health in the large regions of the world where malnutrition is frequently recognized. Even for clinicians working in more prosperous areas valuable information can be extracted. For ten years the authors have jointly been collecting and evaluating the literature in this field. Their final choice of nearly 1500 references tells something about the amount of reading behind this No 57 of the World Health Organization monograph series.

Gunnar Meeuwisse

F Vasella (ed) *Aspekte der pädiatrischen Neurologie* Pädiatrische Fortbildungskurse für die Praxis No 24 113 pp S Karger Basel New York 1968 sFr/DM 26—

This volume is one in a series written for postgraduate courses in paediatrics. It contains good rather

short reviews of some of the actual problems in pediatric neurology. F Vasella Bern has written a very instructive and informative chapter on neurologic examination of infants with tables of motor and mental development. The surveys of cerebral tumours in childhood by E Zander Lausanne and of hydrocephalus by M Bietter Bern are easy to read relatively short but still with all the important aspects on the problems. Other subjects which are reviewed are retinal changes in degenerative disorders of the nervous system acute hemiplegias in childhood leucodystrophies and head injuries. Most of the chapters are followed by a short list of up to date references but some have very few or none at all which is a pity for the interested postgraduate reader.

Ingrid Bjørre

D Jelliffe *Infant Nutrition in the Subtropics and Tropics* Second ed 335 pp illus World Health Organization Geneva 1968 £2 14s

This monograph from WHO was first published 1955 and during the following 13 years there has been a slowly increasing awareness among people in the developed countries of the world of the need for them to participate in the struggle against malnutrition and diseases in the underdeveloped countries. For those who are interested in understanding more about these problems this monograph gives a good introduction. After a short historical survey about infant feeding since ancient civilization there is a presentation of infant feeding practices in about 50 less developed countries all over the third world. Some common patterns of infant feeding are seen in most of the countries e.g. an increased frequency of the failure of lactation among mothers in the lower socio-economic group living in urban areas often associated with unsuccessful attempts at bottle feeding. Animal milk seems to play very little part in the feeding of the majority of poorer infants. Eggs and other protein food are also inadequately used. Carbohydrate in some form is often the sole dominating foodstuff for infants. As a result kwashiorkor and nutritional marasmus often occur and the chapter of the monograph entitled present status of nutritional disease among young children in the subtropics and tropics deals mostly with these two protein calorie malnutrition syndromes. The author gives a short concentrated survey of the syndromes with special emphasis on the importance of early diagnosis and understanding of the miscellaneous factors in their etiology. He also gives recommendations of treatment suitable for large hospitals as well as for rural hospitals. In the chapters dealing with methods

personal style with respect to writing but in spite of this the different parts have many features in common. Thus all main subjects are discussed both from theoretical and clinical point of view and several diagnostic laboratory procedures are included in detail which makes the book useful also as a manual in certain areas. The usefulness of the book is further extended by the large number of references to pertinent literature giving good guidance for those who want to go into more detailed studies of theoretical and/or clinical problems. The balance between theoretical aspects on one hand and clinical and practical on the other is as a rule adequate and in several cases these various aspects are discussed in relation to each other in a way making the presentation methodically entertaining. Most chapters also contain pictures and figures which are most representative and clearly demonstrate that the authors combine theoretical knowledge with practical and clinical experience within their respective fields. It should also be noted that references to literature are most up to date.

This book definitely has its place within the paediatric literature and it can be warmly recommended both to the clinical paediatricians and to scientists within this field. In addition the book should be most useful also to doctors in other fields of medicine in serving as an introduction to clinical endocrinology on the whole.

Berni Hokfelt and Auri Olof Nilsson

L. Dethlefsen, O. Olsson, F. Ström, H. Vreten & A. Zuppinger (eds): *Handbuch der medizinischen Radiologie*. Band X. Teil I. H. Vreten (ed.): *Kontingenzprognostik des Herzes und der Gefäße*. 789 pp. Deutscher Springer Verlag, Berlin, Heidelberg & New York, 1969. DM 296.

This book gives in about 700 pages an extensive description of the roentgen diagnosis of the heart and blood vessels. The topics described in this volume are various techniques of estimation, radiokymographic

the use of contrast media in cardiac catheterization and the physiology, pathophysiology and radiology of heart function and hemodynamics. The illustrations are of high quality. The reference lists are up to date to 1965. The book is of course indispensable for radiologists but can also be recommended to paediatric clinics to be used as a book of reference.

Tor Lindberg

A. Benedikt and P. Csikó: *Zur Frage der Entwicklung von Kindern mit niedrigem Geburtsgewicht*. 167 pp. Akademiai Kiadó Budapest, 1969. US \$6.00.

This book discusses the development of children with low birthweight (less than 2500 g). The study is made on a material of more than 6000 children with low birthweight from Budapest. The first part of the book deals with the late mortality of premature children and points out the rôle of congenital malformation and birth trauma. The second part gives a detailed study of the growth of these children compared with a group of children with normal birthweight and measured by four parameters: body weight, length, chest and head circumference. The results are statistically analysed and a great acceleration in all four parameters is found during the first year of life, mostly pronounced in children with the lowest birth weight. During the second and third year the growth is about the same as for children with normal birth weight. There is no difference between boys and girls but there is evidence that this rapid growth in part depends on the medical care.

There is a short reference list and an extra chapter about the methods of statistical analysis.

The text is in German and rather difficult to read with many tables and mathematic formulas. It could however be recommended to those studying the development of infants with small birthweight because it is a thorough study on a big material and gives a somewhat different aspect on this subject.

Lagard Bjørre



The volume will best serve as an introduction to the field of inborn errors of metabolism its bipolar conception is stimulating Both pediatricians and psychologists/psychiatrists will find it useful

Sture Sjöblad

A J Moss & F H Adams (eds) *Heart disease in infants children and adolescents* The Williams & Wilkins Co. Baltimore 1968 1140 pp illus US \$48.50

During the last decade the field of pediatric cardiology has expanded very rapidly This expansion has made the preparation of a comprehensive pediatric cardiology textbook very difficult for one or a few authors These difficulties have mostly been overcome by publishing a textbook with 73 authors writing in their respective fields

In addition to the description of various acquired and congenital heart diseases the book contains a section of general cardiology with chapters on several subjects such as Fetal circulation and alterations at Birth Genetic aspects of congenital heart disease Electrocardiography etc Other sections contain chapters on special problems such as Systematic hypertension Congenital heart failure and surgical aspects of pediatric cardiology The book is somewhat heterogeneous but most chapters are of high quality while some are excellent The illustrations on the whole are of good quality Some criticism can of course be made i.e. the omission of mentioning the use of  $\beta$  blocking agents in the treatment of cyanotic episodes in tetralogy of Fallot

The book is highly recommended to everyone interested in pediatric cardiology

Nils Rune Lundström

G Sauter (ed) *Kinderpsychiatrie in der Praxis* Psychiatrische Fortbildungskurse für die Praxis Vol 9 S Karger Basel and New York 1968 114 pp DM 24.—

This booklet with its 9 authors in 108 pages gives short informative essays In comparison with the Scandinavian elementary textbooks (A Anell & Lomholt E Regner) it is less systematic and seems to have the ambition to deal with some practically essential topics only often giving priority to therapeutic aspects Some of the authors give concreteness with the help of case reports not aiming at completeness but at demonstration of traits and trends and obvious psychodynamic mechanisms In spite of undisputable instructive value such a limited and condensed selection has its risks To such readers who do not have a rich psychiatric experience of their own it may give the impression of a parade of cases not often seen in reality

The short essays reflect varying attitudes to the conception of psychiatric disease H Stultje builds his

survey of psychoses with regard to disease entities and processes genetic connections developmental stages and special constitutional deviations A Weber describes different neurotic patients mainly from dynamic aspects He also mentions different constitutional disposition to reactions of ambivalence with different risks for neurotic system formation

W Brettschardt writes about moyens thérapeutiques chez des enfants souffrant de lésions cérébrales mineures He gives an instructive and sophisticated short analysis of minimal cerebral lesions and their interaction with psychosocial environment

In a description of therapy to mentally retarded children the mentor in child psychiatry J Lutz gives his skilful apology for wholehearted work for the mentally retarded children and for their need to a humanizing cultivation also in the most desperate cases He gives his experience also of the handling of paternal reactions The main point in his view is the importance of Heilpädagogik No parallels are described to the positive experiences from several other countries of actual efforts to integrate many of the mentally retarded systematically into normal social life away from traditional institutions as much as possible also in towns and cities with regard to living facilities school forms and work forms

It may seem somewhat strange that it was necessary in this actual booklet to warn for the view of glutamic acid as brain food Several authors in the booklet characterize the substance as an effective stimulating and sometimes valuable aid to a passive mind In several countries the substance has for some years been abandoned and is regarded as ineffective for such use

Corboz gives practical hints about quite drastic magic tricks in his essay about medicine to children with deviant behaviour among other things In this connection the question arises to what extent the frequency of patients who dare ask their doctor why might vary in different European countries

This booklet is a complement to the small text books covering more of the whole child psychiatric field It gives references to child psychiatric literature written in German

Ingrid Lagergård

D Hubble (ed) *Pediatric Endocrinology* Blackwell Scientific Publ Oxford 1969 £7 10s

During the last two decades there has occurred a remarkable development within theoretical and clinical endocrinology This has stimulated Do dies Hubble former professor of paediatrics at Birmingham University to initiate the present book aiming at fulfilling a well recognized need within pediatric endocrinology The book contains 10 chapters covering altogether 492 pages Two of these chapters have been written by professor Hubble personally the 8 remaining ones by nine specialists seven representing paediatrics one genetics and one gynecology The various authors have been allowed a great deal of

## FREQUENCY AND NATURE OF RELAPSES IN CHILDREN SUFFERING FROM THE MALABSORPTION SYNDROME WITH GLUTEN INTOLERANCE

J. A. VISAKORPI, P. KUITUNEN and E. SAVILAHTI

From the Children's Hospital, University of Helsinki,  
Helsinki, Finland

According to the literature (2) gluten intolerance appears to exist in several different forms. A typical feature of gluten intolerance in coeliac disease is said to be the disappearance of symptoms and mucosal abnormalities when gluten is avoided and their recurrence when it is ingested again (1). However, it is well known that clinical symptoms of the disease do not always reappear (7) and this has led to the concept of acquired or transient gluten intolerance (2). Investigations based on intestinal biopsy, however, indicate that these patients may undergo relapse without any clinical manifestations (5).

The clinical symptomatology of gluten intolerance has earlier been described by one of us (10) in a group of infants suffering from the malabsorption syndrome. Because the duration of these clinical symptoms was found to be relatively short, it was assumed that most of these patients had transient coeliac disease and they were therefore placed on a gluten-containing diet when they were found to tolerate it clinically.

The purpose of the present follow up study including intestinal biopsy was to throw further

light on the real nature of the gluten intolerance in these patients and to investigate the prognosis of such intolerance.

### MATERIAL AND METHODS

The series comprised 23 patients seen during the years 1962-1964 in whom the malabsorption syndrome with gluten intolerance was diagnosed during the first 2 years of life. Of these 23, 19 were consecutive and non-selected cases which have already been presented by one of us (10). The diagnosis of the malabsorption syndrome was based on the following symptoms: prolonged diarrhoea and/or failure to thrive combined with the finding of an abnormally large amount of faecal fat (more than 4 g/day) and/or abnormally low D-xylose urinary excretion (less than 15%). Gluten intolerance was indicated by a successful response to a gluten free diet within 4 weeks (disappearance of symptoms and gain in weight). Patients with other food intolerances (cow's milk) are not included as this series. The principle of treatment was to keep the patients on a gluten free diet (wheat, rye, oats and barley excluded) only for as long as was necessary to avoid the reappearance of clinical symptoms. Therefore, after clinical improvement on the gluten free diet, was observed, re-introduction of gluten was tried sooner or later. Re-introduction consisted of giving the patient a single dose of 100-200 ml of a wheat-(3%) cow's milk mixture and if no acute reaction was noted the patient was transferred to gluten-containing diet normal for his age. If the patient did not tolerate gluten on this first occasion, re-introduction was tried again later. A follow up study including biopsy was performed on all patients later.

The methods used for investigations are described

Assisted by a grant from the Sigrid Juselius Foundation.

## ANNOUNCEMENT

## SUGGESTIONS CONCERNING THE NOMENCLATURE OF BIRTH WEIGHT AND GESTATIONAL AGE

On Tuesday 7th April 1970 an informal Working Party consisting of Obstetricians and Paediatricians from thirteen European countries who were attending the Second European Congress of Perinatal Medicine met and (after discussion) agreed to make the following suggestions:

(a) That there is a need to define the important groups of babies who present special clinical difficulties and have an increased perinatal mortality in simple words which straightforwardly reflect what can be measured namely birth weight and gestational age (thus excluding all words incorporating maturity which cannot be measured as applied to a whole individual)

(b) That this should make it possible to apply to the *intra uterine* phase of growth the same concepts as have long been applied to the study and definition of growth after birth

(c) That the essential parameter is the gestational age calculated from the menstrual dates (and so perhaps better called menstrual age) Where reliable menstrual information is not available and there is information about the date of ovulation this can be used by adding 14 days to calculate the gestational age. This should then ideally be expressed in days but if weeks are preferred they should be completed weeks (so that 40 weeks=280-286 days) in presenting tables and graphs both the days and the corresponding weeks should be stated. If no information is available it was not felt possible in the present state of knowledge to recommend any of the more technical methods of estimating the gestational age for general use

(d) If it is considered useful (because of differences in mortality and morbidity) to group babies in terms of gestational age the three groups and definitions suggested are

- (i) Pre term less than 259 days (37 weeks)
- (ii) Term 259-293 days (37-41 weeks)
- (iii) Post term 294 days (42 weeks) or more

There was a considerable body of feeling in favour of placing the limit between pre term and term 7 days (1 week) later but the arguments in favour of this were not thought to outweigh the fact that 259 days is the official WHO recommendation (Technical Report Series no 25 1950)

(e) That birth weight is of some practical value in

itself and when it is the only information available horizontal dividing lines of the traditional type may be useful—e.g. 2500 g or less = low birth weight and sub-division by 500 g intervals may also be useful within this low birth weight group. Separate recording of babies with a birth weight of 1000 g or less is in any case useful when making statistical comparisons

(f) The usefulness of birth weight is much increased by relating it to the gestational age and comparing the results with the known distribution in a defined population (e.g. a percentile chart relating birth weight to gestational age preferably sex specific and based upon the population from which the clinical material is drawn—but otherwise upon the best data available for purposes of comparison). The birth weight can then be expressed in appropriate phrases—e.g. below 5th percentile for boys Aberdeen 1963 (or 'light for dates')

## Members of the Working Party

Chairman Dr G. A. Neligan U.K.

Members: Dr A. Ballabriga Spain Professor H. Beitzel vice Professor H. Wulf Germany Professor Buca Italy Dr P. M. Dunn U.K. vice Professor N. E. Butler Professor H. Ewerbeck Germany (*Abstrakt für Kinderheilkunde Zentralblatt für Kinderheilkunde*) Dr B. Fnu Hansen Denmark (Member Ed. Board of *Acta Paediatrica Scandinavica*) Member Ed. Board of *Pediatric Research* Dr D. Gardner U.K. (Editor *Archives of Diseases in Childhood*) Dr J. Geatz vice Professor R. Zetterstrom Sweden (Editor *Acta Paediatrica Scandinavica*) Professor P. O. Hubonot Belgium Professor P. Karlberg Sweden Professor G. J. Kloosterman Holland Dr F. Kubli Switzerland Professor A. A. Minkowski France (Editor *Biologie Neonatorum*) Editor *Developmental Psychology* Professor L. S. Prodhon Switzerland Dr G. Rooth Sweden Professor E. Salang Germany Professor C. Sureau France (Editor in Chief *Gynecologie et Obstetrique*) Member of the Editorial Board of the *International Journal of Obstetrics and Gynecology* Dr T. Vabes Greece Professor J. Horáky Czechoslovakia Secretary Professor P. J. Huntingford U.K. (As assistant Editor of the *Journal of Obstetrics and Gynecology of the British Commonwealth*)

Table 2 Symptoms and laboratory data in the eight symptomatic relapses at the time of the relapse\*

Diarrhoea	7/8
Vomiting	2/8
Failure to gain weight	8/8
Haemoglobin under 11 g/100 ml	3/5
Serum iron under 40 µg/100 ml	5/5
Fat excretion more than 4 g/day	1/4
D-xylose excretion less than 15"	1/4
Positive urinary FIGLU	4/4
Preprecipitate to cow's milk	3/4
Preprecipitate to gluten	4/4
Elevated serum IgA	3/4
Small intestinal mucosa	
— Subtotal villous atrophy	4/4

\*Only four of the symptomatic relapses could be studied by us before return to the elimination diet. The remaining four were studied later and in all the small intestinal mucosa was abnormal at the time of examination.

tion diet. During this period eight of the 23 patients underwent a clinically evident relapse whereas the other 15 continued to be symptom free. In all these eight relapses the diagnosis was verified by finding an abnormal intestinal mucosa at the time of the relapse or later. All 15 asymptomatic children were also biopsied and in 14 an abnormal mucosa was found.

**Symptomatic relapses.** Symptomatic relapse became apparent soon after reintroduction of wheat, the average time being 6.4 months after the return to a normal diet. As the data in Table 1 indicate there was not much difference either in the initial clinical picture or in the course of the disease between the symptomatic and asymptomatic relapses. When a test for gluten intolerance was made during the initial elimination diet period only 1 of these 8 patients reacted. The symptoms in relapses (Table 2) were the same as in the initial phase of the disease although milder. In 1 case a change in the growth pattern was the only symptom. The results of absorption tests, immunological examination and biopsy were also abnormal.

Clinically all relapses responded well to reintroduction of the gluten free diet. Three of these 8 patients were again treated only by a short course of the elimination diet and have since then been asymptomatic but with an ab-

normal mucosa. The other 5 patients are being treated continuously; the mucosa has shown complete normalisation in one (at 3.3 years) and improvement in three (at 2.4, 3.6 and 3.8 years) whereas in 1 patient comparison is impossible because the initial biopsy at the relapse did not succeed.

**Asymptomatic relapses.** As mentioned earlier 14 patients were clinically asymptomatic and growing normally during the whole observation period, the small bowel mucosa being clearly abnormal in each case. The average time for which these 14 asymptomatic patients were observed after resumption of a normal diet was 3.7 years (range 1.9–6.5 years). The results of laboratory tests made on these patients in connection with the follow up study are presented in Table 3. Two of the patients had an abnormal D-xylose excretion test and one of these also had an abnormally large amount of faecal fat. Immunological tests also gave abnormal results in some patients.

Two non selected asymptomatic patients were later placed on a gluten free diet again. One of these had actually only been treated for 1.5 months being then asymptomatic for 2.2 years although having a flat mucosa. On a long term gluten free diet the mucosa was found to have become normal after 2.4 years. Rein-

Table 3 Data of the 14 asymptomatic relapses at the last follow up examination on a normal gluten containing diet

Weight above 16 percentile	11/14
Weight above 2.5 percentile	14/14
Height above 16 percentile	12/14
Height above 2.5 percentile	14/14
Normal bone age	6/8
Haemoglobin under 11 g/100 ml	4/14
Serum iron under 40 µg/100 ml	2/12
Fat excretion more than 4 g/day	1/10
D-xylose excretion less than 15"	2/12
Positive urinary FIGLU	0/9
Preprecipitate to cow's milk	6/11
Preprecipitate to gluten	3/11
Elevated serum IgA	3/11
Small intestinal mucosa	
— Subtotal villous atrophy	11/14
— Partial villous atrophy	3/14

All were within two standard deviations and distribution was normal.

Table 1 Data about the course of the disease main initial symptoms and findings at the time of diagnosis in different groups in the study

	All	Symptomatic relapsers	Asymptomatic relapsers	Recovered
Number of patients	23	8	14	1
Male/female	11/12	4/4	6/8	1/0
Age at onset of symptoms (mean and range) in months	6.5 (4-18)	7.8 (4-18)	6.0 (4-10)	4
Age at time of diagnosis (mean and range) in months	9.7 (5-24)	10.2 (5.5-24)	9.7 (5.0-18)	5
Duration of gluten containing diet before diagnosis (mean and range) in months	6.9 (1-19)	6.7 (2.3-19)	7.5 (7.5-14)	1
Duration of initial treatment with gluten free diet (mean and range) in months	9.7 (0.6-25)	11.8 (2-25)	7.5 (0.6-19)	24
Duration of gluten containing diet before relapse or before last examination (mean and range)	2.6 (0.16-6.5) years	6.4 (2-18) months	3.7 (1.9-6.5) years	4.6 years
Prolonged diarrhoea	21/23	7/8	13/14	1/1
Vomiting	20/23	6/8	13/14	1/1
Failure to gain weight	23/23	8/8	14/14	1/1
Weight under 2.5 percentile	17/23	6/8	10/14	1/1
Weight under 16 percentile	23/23	8/8	14/14	1/1
Enlarged abdomen	15/23	4/8	10/14	1/1
Haemoglobin under 10 g/100 ml	7/23	3/8	4/14	0/1
Serum iron under 40 µ/100 ml	15/21	5/8	9/12	1/1
Fat excretion more than 4 g/day	21/22	8/8	12/13	1/1
D-xylose excretion less than 15	18/22	7/7	10/14	1/1
Positive urinary FIGLU	18/22	5/7	12/14	1/1
Precipitins to cow's milk	16/16	4/4	11/11	1/1
Precipitins to gluten	11/16	3/4	8/11	0/1
Elevated serum IgA	10/16	4/4	5/11	1/1
Small intestinal mucosa				
— Subtotal villous atrophy	12/13	3/3	9/10	0/0
— Partial villous atrophy	1/13	0/3	1/10	0/0

earlier (3.4.11). Bone age was estimated according to the method described by Tanner *et al* (9).

#### Description of the patients

The average age of the patients at the time of diagnosis was 9.7 months and the mean duration of symptoms before admission 3.2 months. The main symptoms and signs as well as the results of the diagnostic laboratory tests are given in Table 1. During the first 12 months after the start of the diet reintroduction of gluten was tried in 21 cases whereas in 2 cases the first attempt was only made after 2 years treatment for reasons not dependent on the clinical picture. In the above mentioned 21 cases reintroduction was successful in 14 patients whilst 7 patients gave a clinical reaction to gluten. The second attempt at reintroduction was successful in all these 7. Altogether the first reintroduction was successful in 16 cases and the second in all the remaining 7 cases.

The mean duration of the initial gluten free diet

was 9.7 months (range 0.6-25 months). But this can not be taken as the length of the period during which the patients were intolerant to gluten because the last moment when they were still sensitive to gluten is not known precisely.

At the time of completion of the diet every patient was in good clinical condition. Eight patients had reached a normal weight while the others were still catching up. In all the 8 patients tested the absorption function was normalized. No biopsy was performed at the time of transfer to the normal diet.

#### RESULTS

**General outlines** The average length of the follow up period until the last examination or until the patients were placed permanently on a gluten free diet was 2.6 years (range 0.2-6.5 years) after completion of the initial elimina-

no significant differences could be detected in the primary symptomatology in the clinical severity of the disease in the results of laboratory tests or in the response to elimination of gluten. As a matter of fact the duration of the diet was longer in the asymptomatic relapsers but it is difficult to evaluate the significance of this observation because the duration of the diet was often dependent on irrelevant factors. Most of the clinical relapses occurred within a short while after reintroduction of gluten and patients who had been asymptomatic for one year seemed to remain permanently symptom free.

Is it possible to detect these asymptomatic relapses without a biopsy? At least the tests used in this study failed to do so. The highest incidence of abnormal results was found in the immunological tests but even these were normal in some cases.

One might now ask whether this mucosal damage in the asymptomatic patients is really gluten induced. We believe this to be the case although the causal relationship has not been directly confirmed in this study. It has been shown in at least one of our asymptomatic patients who has developed a normal mucosa when returned to a gluten free diet. Since the year 1964 all similar patients have been treated by us with long term gluten free diet, and with this latter group it has been possible to demonstrate convincingly that the mucosal lesion really is gluten induced. The results of this study will be reported in due course.

However in the present series there was 1 patient who probably recovered completely. Clinically this patient was a typical case of coeliac disease even showing clinical symptoms in a provocation test. Unfortunately the initial biopsy was unsuccessful and this means that we do not possess incontrovertible proof of the existence of transient coeliac disease. Nevertheless this case suggests that transient clinical intolerance to gluten does occur. Further information on similar cases with full series of biopsies are needed.

## SUMMARY

This is the report of a follow up study of 23 children who in infancy suffered from the malabsorption syndrome with gluten intolerance. These patients had been treated with gluten elimination only for the short period during which they were clinically intolerant to gluten. Three patients tolerated gluten after elimination for as little as 2 months but the mean duration of elimination was 9.7 months. During the observation period 8 patients developed symptomatic relapse mostly soon after reintroduction of gluten into the diet whereas 15 continued to develop normally and were asymptomatic. However 14 of these 15 were found to have an abnormal intestinal mucosa.

In 3 cases the symptomatic relapses were treated by a further short period on an elimination diet after which the patients showed normal development although the mucosa remained abnormal. Five of the relapsers were treated with long term elimination and improvement of the intestinal mucosa was observed in all of them. The only way to detect the asymptomatic patients was by biopsy because the absorption tests and immunological tests failed to reveal most of them.

The only patient who was completely normal in the follow up study after consuming normal gluten-containing food for 4.6 years was initially a typical case of coeliac disease also reacting to gluten in a provocation test but unfortunately the diagnosis had not been verified by an initial biopsy.

## REFERENCES

- Anderson C. M. Intestinal malabsorption in childhood. *Arch. Dis. Child.* 41: 571 1966.
- Fruzer A. C. *Malabsorption syndromes*. William Heinemann Medical Books Ltd. London 1968.
- Immonen P. Levels of serum immunoglobulins gamma G, gamma O and gamma M in the malabsorption syndrome in children. *Ann. Paediatr. Fenn.* 13: 115 1967.
- Kontinen H. Duodeno-jejunal histology in malabsorption syndrome in infants. *Ann. Paediatr. Fenn.* 12: 101 1966.

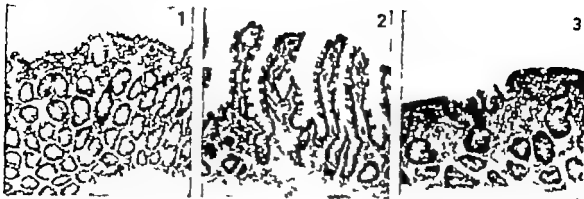


Fig 1 Small intestinal follow up biopsies of a boy on whom the diagnosis was made at the age of 7 months. Primary biopsy showed clearly abnormal mucosa but the slides are technically poor. The boy was kept for 15 months on gluten free diet after which he took normal food for 22 years and was

doing well although biopsy (1) revealed a completely flat mucosa. The boy was placed on gluten free diet and the mucosa normalised in 24 years (2). When he was exposed to gluten again for 7 months the violent atrophy recurred (3).

roduction of gluten after this caused deterioration of the mucosa again in 7 months (Fig 1). In the other asymptomatic patient the mucosa had not normalised when examined 23 years after elimination of gluten.

**Non relapsing patient** Only 1 patient was completely normal in the follow up study. This patient was admitted for the first time at the age of 5 months having had wheat in the diet for 1 month. The symptoms were typical, faecal fat excretion 6.8 g/day and D xylose excretion test 14% but no initial biopsy was taken. The patient responded well to a gluten-free diet and a provocation test with gliadin was positive (vomiting and loss of weight). This patient was treated for 2 years with gluten elimination. The follow up examination was performed 46 years after reintroduction of gluten. At this point the patient was normal in every respect including absorptive function and mucosal morphology.

## DISCUSSION

In an earlier report concerning the initial phase of the disease in these patients special attention was drawn to the relatively short duration of the gluten intolerance in some cases. It was suggested that these patients might have only a secondary intolerance to gluten. The results of this follow-up study show that this was not

the case but that all but apparently one of the patients were suffering from true coeliac disease.

This report also shows as did for example, the results published by Sheldon (7) and Mortimer *et al* (5) that a great number of patients develop a clinically "silent" state after the initial treatment. It is surprising how short an elimination period may suppress the active clinical symptomatology. 2 months or less was enough in 3 cases. We feel that these patients of ours are unlikely to be exceptional in this respect, because they were unselected and consecutive. The early age of our patients and the short duration of their disease before treatment may be one factor reducing the duration of the period of active gluten intolerance.

These results also expose the fallacy of basing the diagnosis of coeliac disease on reappearance of the symptoms when gluten consumption is resumed because it is always possible that at the time when the provocation test was performed the period during which the patient would have shown clinical symptoms of sensitivity to gluten had already passed. A biopsy is at present the only known way to establish whether gluten intolerance is permanent.

Are there then primarily any differences between patients who exhibit symptomatic relapse and those who do not? In our series

no significant differences could be detected in the primary symptomatology in the clinical severity of the disease in the results of laboratory tests or in the response to elimination of gluten. As a matter of fact the duration of the diet was longer in the symptomatic relapsers but it is difficult to evaluate the significance of this observation because the duration of the diet was often dependent on irrelevant factors. Most of the clinical relapses occurred within a short while after reintroduction of gluten and patients who had been asymptomatic for one year seemed to remain permanently symptom free.

Is it possible to detect these asymptomatic relapses without a biopsy? At least the tests used in this study failed to do so. The highest incidence of abnormal results was found in the immunological tests but even these were normal in some cases.

One might now ask whether this mucosal damage in the asymptomatic patients is really gluten induced. We believe this to be the case, although the causal relationship has not been directly confirmed in this study. It has been shown in at least one of our asymptomatic patients who has developed a normal mucosa when returned to a gluten free diet. Since the year 1964 all similar patients have been treated by us with long term gluten free diet and with this latter group it has been possible to demonstrate convincingly that the mucosal lesion really is gluten induced. The results of this study will be reported in due course.

However in the present series there was 1 patient who probably recovered completely. Clinically this patient was a typical case of coeliac disease even showing clinical symptoms in a provocation test. Unfortunately the initial biopsy was unsuccessful and this means that we do not possess incontrovertible proof of the existence of transient coeliac disease. Nevertheless this case suggests that transient clinical intolerance to gluten does occur. Further information on similar cases with full series of biopsies are needed.

## SUMMARY

This is the report of a follow up study of 23 children who in infancy suffered from the malabsorption syndrome with gluten intolerance. These patients had been treated with gluten elimination only for the short period during which they were clinically intolerant to gluten. Three patients tolerated gluten after elimination for as little as 2 months but the mean duration of elimination was 9.7 months. During the observation period 8 patients developed symptomatic relapse mostly soon after reintroduction of gluten into the diet whereas 15 continued to develop normally and were asymptomatic. However 14 of these 15 were found to have an abnormal intestinal mucosa.

In 3 cases the symptomatic relapses were treated by a further short period on an elimination diet after which the patients showed normal development, although the mucosa remained abnormal. Five of the relapsers were treated with long term elimination and improvement of the intestinal mucosa was observed in all of them. The only way to detect the asymptomatic patients was by biopsy because the absorption tests and immunological tests failed to reveal most of them.

The only patient who was completely normal in the follow up study after consuming normal gluten-containing food for 4.6 years was initially a typical case of coeliac disease also reacting to gluten in a provocation test but unfortunately the diagnosis had not been verified by an initial biopsy.

## REFERENCES

- 1 Anderson C M. Intestinal malabsorption in childhood. *Arch Dis Child* 41: 571 1966.
- 2 Frazer A C. *Malabsorption syndromes*. Williams & Heinemann Medical Books Ltd. London 1968.
- 3 Imeson P. Levels of serum immunoglobulins gamma A, gamma G and gamma M in the malabsorption syndrome in children. *Ann Paediatr Fenn* 13: 115 1967.
- 4 Kuitman P. Duodeno-jejunal histology in malabsorption syndrome in infants. *Ann Paediatr Fenn* 12: 101 1966.





Fig 1 Small intestinal follow up biopsies of a boy on whom the diagnosis was made at the age of 7 months. Primary biopsy showed clearly abnormal mucosa but the slides are technically poor. The boy was kept for 15 months on gluten free diet after which he took normal food for 22 years and was

doing well although biopsy (1) revealed a completely flat mucosa. The boy was placed on gluten free diet and the mucosa normalised in 24 years (2). When he was exposed to gluten again for 7 months the villous atrophy recurred (3).

roduction of gluten after this caused deterioration of the mucosa again in 7 months (Fig 1). In the other asymptomatic patient the mucosa had not normalized when examined 23 years after elimination of gluten.

*Non relapsing patient* Only 1 patient was completely normal in the follow up study. This patient was admitted for the first time at the age of 5 months having had wheat in the diet for 1 month. The symptoms were typical: faecal fat excretion 6.8 g/day and D-xylose excretion test 14% but no initial biopsy was taken. The patient responded well to a gluten-free diet and a provocation test with gliadin was positive (vomiting and loss of weight). This patient was treated for 2 years with gluten elimination. The follow up examination was performed 46 years after reintroduction of gluten. At this point the patient was normal in every respect including absorptive function and mucosal morphology.

## DISCUSSION

In an earlier report concerning the initial phase of the disease in these patients special attention was drawn to the relatively short duration of the gluten intolerance in some cases. It was suggested that these patients might have only a secondary intolerance to gluten. The results of this follow up study show that this was not

the case but that all but apparently one of the patients were suffering from true coeliac disease.

This report also shows as did for example the results published by Sheldon (7) and Mortimer *et al* (5) that a great number of patients develop a clinically silent state after the initial treatment. It is surprising how short an elimination period may suppress the active clinical symptomatology: 2 months or less was enough in 3 cases. We feel that these patients of ours are unlikely to be exceptional in this respect, because they were unselected and consecutive. The early age of our patients and the short duration of their disease before treatment may be one factor reducing the duration of the period of active gluten intolerance.

These results also expose the fallacy of basing the diagnosis of coeliac disease on reappearance of the symptoms when gluten consumption is resumed, because it is always possible that at the time when the provocation test was performed the period during which the patient would have shown clinical symptoms of sensitivity to gluten had already passed. A biopsy is at present the only known way to establish whether gluten intolerance is permanent.

Are there then primarily any differences between patients who exhibit symptomatic relapse and those who do not? In our series

## UNILATERAL NEUROLOGICAL DEFECT IN MYELOMENINGOCELE WITH NORMAL BLADDER FUNCTION

### Report on Two Cases

N O ERICSSON B HELLSTRÖM A NERGÅRDH and U RUDHE

From the Department of Paediatrics Karolinska sjukhuset Stockholm Sweden

Various clinical reviews concerning myelomeningoceles give the incidence of normal urinary control as between 3% (4) and 33% the latter figure originating from a somewhat selected series (3). With the cecum in lumbosacral region the continence figures are generally low. The criteria of continence and particularly of normal bladder function vary. Sometimes the reports are based entirely on the history, sometimes bladder function is studied in a more detailed and objective manner. Only in recent years have conditions for the development of entirely intact bladder function begun to arise. Greater interest, knowledge of these should estimate the basis of any clinical attempt to have continence. We shall here describe 2 cases of myelomeningocele which developed normal bladder function.

These 2 cases were among the last 50 consecutive patients with myelomeningocele in the lower thoracic or the lumbosacral areas who were referred for urological evaluation. This group was examined by a team of a neurologist, a radiologist and a urologist.

### CASE REPORTS

**Case 1** Boy with a lumbosacral myelomeningocele at the age of 3½ weeks transferred to the neurosurgical clinic. Report states that the boy moved his

This work has been supported by a grant from Svenska Läkaresällskapet. Named for Medicinsk Forskning Stockholm.

legs well and had anal sphincter function. No hydrocephalus. At operation nerve fibres were found passing distally into "indifferent" tissue in the lower right part of the sac. No diastematomyelia was present. The sac was excised. The postoperative course was uneventful.

Psychomotor development was normal until the age of 1 year when it was noted that the patient had difficulty in standing with support. His left leg was somewhat shorter than the right. With an orthopedic splint he began to walk and run although with a limp.

He developed control of urines and faeces at the age of 1½ months but at 2 years of age began to develop encopresis and diurnal enuresis. At the same time and during the following years his home environment worsened considerably because his mother became paranoid.

The patient was referred for urological investigation at the age of 8 years. He showed complete control of urination and defecation. Normal stream on micturition. No leakage of urine on bladder expression. Normal urinalyses. Residual urine 5 ml. Normal renal function and cystometrogram.

**Neurological investigation:** Left leg 2 cm shorter than the right. On the left side decreased muscular strength in hip extensors, upward rotators and knee flexors. Atrophy of musculature of the lower leg (Fig. 1). Complete paralysis of the muscles of the foot and toes. Knee jerks normal bilaterally. Achilles and plantar reflexes absent on left side. Loss of cutaneous and temperature sensation corresponding to involvement of the first sacral segment and partial involvement of the fifth lumbar segment on the left side. Anocutaneous reflex normal. Normal anal and sphincter tone. X-ray showed defects of the arches of the 4th and 5th lumbar vertebrae. Intravenous urography and micturating cystourethrography were normal. No vesico-urethral reflux was present.

**Case 2** Girl with skin-covered lumbosacral myelomeningocele. Referred the first time on the

- 5 Mortimer P E, Stewart J S, Norman A P & Booth C C. Follow up study of coeliac disease. *Brit Med J* 3 7 1968
- 6 Rubin C E. Celiac disease and idiopathic sprue. Some reflections on reversibility, gluten and the intestine. *Gastroenterology* 39 260 1966
- 7 Sheldon W. Celiac disease. *Pediatrics* 23 132 1959
- 8 Shmerling D H. An analysis of controlled relapses in gluten induced coeliac disease. *Abst European Soc Ped Gastroenterology* 1968
- 9 Tanner J M, Whitehouse R H & Healy M J R. A new system for estimating the maturity of the hand and wrist with standards derived from 2600 healthy British children. Part II. The scoring system. *International Children's Centre Paris* 1962
- 10 Visakorpi J K & Immonen P. Intolerance to cows milk and wheat gluten in the primary malabsorption syndrome in infancy. *Acta Paediatr Scand* 56 49 1967
- 11 Visakorpi J K, Immonen P & Kuitunen P. Malabsorption syndrome in childhood. The occurrence of absorption defects and their clinical significance. *Acta Paediatr Scand* 56 1 1967

Submitted Dec 4 1969

Accepted March 12 1970

(J K V) Children's Hospital  
Stenbackinkatu 11  
Helsinki 29  
Finland

**Key words:** Coeliac disease, malabsorption syndrome, gluten

moderate decrease in ability to move the foot and toes and unchanged left-sided atrophy of the calf muscles (Fig. 2) and absent Achilles and plantar reflexes. Decreased superficial sensation corresponding to the three lowest sacral segments dermatome on the left side as well as partially corresponding to the first sacral segment dermatome on the right side. Normal anal sphincter tone. Normal intravenous pyelogram. Micturition cystography showed a bladder of normal capacity and demonstrated the previously noted asymmetry of the bladder neck. The urethra was entirely normal and showed no sign of abnormal sphincter activity. No reflux to upper urinary tract could be demonstrated.

## DISCUSSION

In both of these cases there was a cystic spinal bifida in the lumbosacral region with clear evidence of lots of neurological function in cheating myelomeningocele. In both patients function loss corresponded to involvement of L<sub>4</sub>-S<sub>2</sub> as the upper limit. There was evidence of motor and sensory impairment as well as loss of reflexes but almost solely on the left side. In both cases satisfactory bladder function was present as regards both detrusor and sphincter function. Anal sphincter function was also normal. Symptoms of enuresis and encopresis of psychogenic origin were present in the first patient.

In case 2 there was uro-radiological evidence of some changes in the bladder neck which showed an abnormal configuration during micturition characterized by right sided widening. This remained a constant feature during subsequent examinations from the age of 1 week to 6 years. It was noteworthy that this alteration primarily affected the right side where the loss of neurological function was hardly evident. Moreover the left portion of the bladder neck repeatedly was found to be normal despite the presence of serious skeletal defects at the level of the sacral myelomeningocele on the same side.

In most cases of myelomeningocele bladder function is altered. Although exceptions have been noted the conditions necessary for retaining a certain level of bladder innervation have for the most part, seldom been discussed

in the literature. In this connection the level of the lesion is of some importance. When the sac is situated in the cervical or upper thoracic spine neurological defects may be minimal and bladder function more or less normal (3). These locations however are relatively unusual (4). In cases of myelomeningocele involving the lower portion of the thoracic or thoraco-lumbar cord some function often remains in regions caudal to the level of the lesion—i.e. distal spasticity in the lower extremities or an intact anocutaneous reflex. In such cases bladder alterations of upper motor neuron type with reflexinhibited partial or complete bladder-emptying may occur. An intact reflex activity within the sacral cord can also lead to a hyperactive external sphincter which in turn may produce apparent partial continence. In the long run however this is functionally unsatisfactory as "pseudo-continence" is accompanied by residual urine and involves a threat to kidney function.

The importance of intact reflex activity in the 2nd to 4th segment of the sacral cord for partial detrusor function has recently been clarified by Stark (5). It seems possible that this reflex activity is more related to bladder function than the upper level of the lesion. In his series there are some patients with normal bladder function and a number of them had diastematomyelia with unilateral lesion of the spinal cord. Similar cases have also recently been described by Dockworth *et al.* (2) who on surgical exploration found a hemimyelocoele with preponderant unilateral dysplasia. Moreover the majority of patients had for the most part, intact bladder function although a more detailed uro-radiological investigation was not published. Progressive scoliosis was an important feature accompanying the hemimyelocoele. This was not a prominent feature in our cases nor was an anatomical hemimyelocoele found in the case operated upon. Furthermore no sign of osseous diastematomyelia could be shown on roentgenograms. Apart from the above mentioned studies there appears to be only one other re-

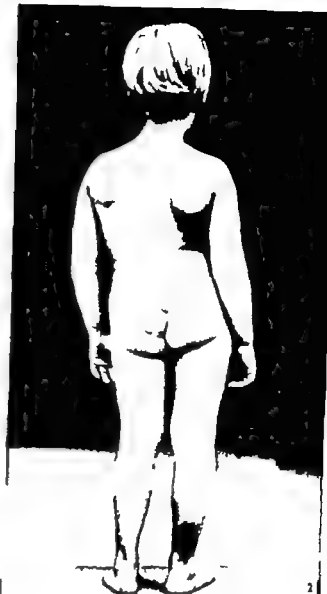


Fig 1 and 2 Patients presented in the text. In both cases asymmetry of the neurological defect is appar-

ent with slight shortness of left leg and moderate atrophy of lower left leg

sixth day of life. She was noted to have her legs flexed at hips but no definite neurological abnormalities were noted.

Röntgenological studies showed severe defects in the arches of the 4th to 5th lumbar vertebrae, hypoplasia of the left aspects of all five sacral vertebrae and of the right aspect of  $L_4$  as well as abnormal diastasis between the vertebral bodies and the rest of the vertebrae. Intravenous pyelogram was normal. Micturating urethrocytography showed a normal bladder. During micturition there was reflux to the right ureter and kidney pelvis without dilatation. The bladder neck was asymmetrically wider than normal on the right side. A polyp was present in the proximal urethra which was otherwise normal.

The urethral polyp was resected transurethrally. The sacral tumour was not operated on and was considered to be a meningo- or a myelomeningocele. There was no hydrocephalus.

Examinations at the ages of 4 and 9 months showed no change in the findings. At 15 months the patient began to walk and it was noted that she lumped. The left leg was 2 cm shorter than the right. On the left side there was a moderate degree of atrophy of the calf musculature and an absent Achilles reflex. Bladder function was good. Residual urine 4 ml. On two occasions moderate bacteriuria but no clinical evidence of urinary infection. Complete control of both bladder and rectum. Sensation of bladder and rectal filling remained intact. Bladder neck showed the same asymmetrical shape as previously. Bilateral vesico-ureteral reflux now present.

The last examination took place at the age of 6 years. The patient was then completely continent. Micturition normal. No leakage of urine on bladder expression. No residual urine. Normal urinalysis without bacteriuria. Normal renal function. On the left side some decrease in strength of knee flexors.

moderate decrease in ability to move the foot and toes and unchanged left-sided atrophy of the calf muscles (Fig. 2) and absent Achilles and plantar reflexes. Decreased superficial sensation corresponding to the three lowest sacral segments dermatomes on the left side as well as partially corresponding to the first sacral segments dermatomes on the right side. Normal anal sphincter tone. Normal intravenous pyelogram. Micturating cystourethrography showed a bladder of normal capacity and demonstrated the previously noted asymmetry of the bladder neck. The urethra was entirely normal and showed no sign of abnormal sphincter activity. No reflex to upper sensory tract could be demonstrated.

## DISCUSSION

In both of these cases there was a cystic spinal bulgia in the lumbosacral region with clear evidence of loss of neurological function in treating myelomeningocele. In both patients function loss corresponded to involvement of  $L_4-S_1$  as the upper limit. There was evidence of motor and sensory impairment as well as loss of reflexes but almost solely on the left side. In both cases satisfactory bladder function was present as regards both detrusor and sphincter function. Anal sphincter function was also normal. Symptoms of contusis and encopresis of psychogenic origin were present in the first patient.

In case 2 there was uroadiological evidence of some changes in the bladder neck which showed an abnormal configuration during micturition characterized by right sided widening. This remained a constant feature during subsequent examinations from the age of 1 week to 6 years. It was noteworthy that this alteration primarily affected the right side where the loss of neurological function was hardly evident. Moreover the left portion of the bladder neck repeatedly was found to be normal despite the presence of serious skeletal defects at the level of the sacral myelomeningocele on the same side.

In most cases of myelomeningocele bladder function is altered. Although exceptions have been noted the conditions necessary for retaining a certain level of bladder innervation have not been fully discussed. The latter part seldom been discussed

in the literature. In this connection the level of the lesion is of some importance. When the sac is situated in the cervical or upper thoracic spine neurological defects may be minimal and bladder function more or less normal (3). These locations however are relatively unusual (4). In cases of myelomeningocele involving the lower portion of the thoracic or thoraco-lumbar cord some function often remains in regions caudal to the level of the lesion—i.e. distal spasticity in the lower extremities or an intact anocutaneous reflex. In such cases bladder alterations of "upper motor neuron type" with reflexinhibited partial or complete bladder-emptying may occur. An intact reflex activity within the sacral cord can also lead to a hyperactive external sphincter which in turn may produce apparent partial continence. In the long run however this is functionally unsatisfactory as "pseudo-continence" is accompanied by residual urine and involves a threat to kidney function.

The importance of intact reflex activity in the 2nd to 4th segment of the sacral cord for partial detrusor function has recently been clarified by Stark (5). It seems possible that this reflex activity is more related to bladder function than the upper level of the lesion. In his series there are some patients with normal bladder function and a number of them had diastematomyelia with unilateral lesion of the spinal cord. Similar cases have also recently been described by Duckworth *et al* (2) who on surgical exploration found a hemimyelomeningocele with preponderant unilateral dysplasia. Moreover the majority of patients had for the most part, intact bladder function although a more detailed uroadiological investigation was not published. Progressive scoliosis was an important feature accompanying the hemimyelomeningocele. This was not a prominent feature in our cases nor was an anatomical hemimyelomeningocele found in the case operated upon. Furthermore no sign of osseous diastematomyelia could be shown on roentgenograms. Apart from the above mentioned studies there appears to be only one other re-

port describing a few cases of asymmetrical innervation all of which had normal sphincter control (1).

The cases here described confirm the view that when myelodysplasia with cystic spina bifida primarily causes unilateral neurological disturbance, function may be sufficiently retained within the 2nd to 4th sacral segment to permit entirely normal bladder and anal sphincter activity.

The true incidence of such asymmetrical defects cannot be determined from our series. Less evident asymmetries, particularly those concerning sensory disturbances are common. Frequently there is a difference between both sides as regards the extent of the lesions, which may involve one or two segment levels.

More account should be taken of the minority of cases with myelomeningocele which have partial or completely normal bladder function. Increased knowledge of such cases can further clarify studies of incontinence. This in turn, can lead to a more rational approach to the conquest of this very troublesome social handicap.

### SUMMARY

Two cases of myelomeningocele with symptoms of unilateral disturbance and with normal bladder function are discussed.

### REFERENCES

- 1 Doran P A & Guthkelch A N Studies in spina bifida. Part IV. The frequency and extent of paralysis. *J Neurol Neurosurg Psychiatr* 26: 545 1963.
- 2 Duckworth T, Sharrard W J, Lister J & Seymour N. Hemimyelomeningocele. *Develop Med Child Neurol Suppl* 16: 69 1968.
- 3 Eckstein H B. Urinary control in children with myelomeningocele. *Brit J Urol* 40: 191 1968.
- 4 Smith, E D. *Spina bifida and the total care of spinal myelomeningocele*. Charles C Thomas, Springfield, Ill 1965.
- 5 Stark G. Pathophysiology of the bladder in myelomeningocele and its correlation with the neurological picture. *Develop Med Child Neurol Suppl* 16: 76 1968.

Submitted Dec 8 1969

Accepted Jan 8 1970

(N O E) Dept of Paediatrics  
Karolinska Sjukhuset  
104 01 Stockholm 60  
Sweden

**Key words:** Myelomeningocele, normal bladder function, unilateral neurological defect.

## FACTORS PROMOTING URINARY AND ANAL CONTINENCE IN CHILDREN WITH MYELOMENINGOCELE

N O ERICSON B HELLSTRÖM A NERGÅRDH and U RUDHE

*From the Department of Paediatrics Karolinska sjukhuset Stockholm Sweden*

In a previous study (5) 2 cases of myelomeningocele with normal bladder function were described. In these patients the clinical picture was characterised by unilateral disturbance of neurological function. They were included in a recent series of 50 cases investigated neurologically, radiologically and urologically. The degree of severity and the type of incontinence or partial continence varied. In the literature, however, opinions are found concerning the relationship between remaining bladder function and the neurological findings.

In this study an attempt has been made using ordinary clinical methods to determine the conditions for acquisition of partial bladder and anal continence. The aim has been to select suitable cases which might respond to bladder training, either partially or completely and thus permitting the social rehabilitation of the patient. The literature on this subject is scanty.

### MATERIAL

The case material consists of 24 boys and 26 girls who were referred for urological investigation. The majority were admitted to the hospital on more than one occasion and examined regularly twice a year. Some have been studied from the newborn period. At the time of the last examination their ages ranged from 2 to 16 years. The degree of continence has

been determined from the history and from the clinical observations as regards habitual and occasional dry periods.

The micturition history has included information on the patient's ability to experience the sensations of bladder filling and a desire to void as well as the attention involved in voiding. The ability to initiate micturition has been noted and whether this has occurred with or without increasing the intra-abdominal pressure. The type of urinate and the patient's capacity to voluntarily interrupt micturition have also been recorded. During hospitalisation micturition has been studied in detail and compared with the information given in the history. Analyses has invariably included an investigation of the response of the bladder to expression, measurement of residual urine after spontaneous micturition or expression, urine analyses, including quantitative bacteriological culture, serum osmolality and serum creatinine or blood urea nitrogen. In some cases cystometry and kidney function tests were carried out. Intravenous urography and micturition cystoscintigraphy were regularly performed.

Anal function was evaluated on the basis of the history and hospital records as regards continence and its relationship to the coexistence of stools. The patient's ability to experience the desire to defecate on filling of the ampulla was noted. The physical examination included inspection of the anal region, evaluation of anal sphincter function by palpation and investigation of the anocutaneous reflex. Roentgenologic studies of defecation were performed in 9 cases. The rectum was filled with thick barium contrast medium and frontal and lateral views were taken. In several cases the patient's ability to voluntarily elevate his pelvic floor was studied.

The neurological evaluation included demonstration of motor and sensory loss as well as cutaneous and tendon reflexes. On the basis of these findings the patients were grouped according to the neurological level present of the various lesions. Special attention was paid to the occurrence of preserved

This work has been supported by a grant from Svenska Livförsäkringsbolagets Nämnd för Medicinsk Forskning Stockholm.



functions caudal to the level of the lesion. Neurological classification according to the upper level of the functional lesion resulted in the following:

*Group 1 (low thoracic)* Complete paraplegia was found sometimes accompanied by loss of strength in the trunk and particularly the abdominal muscles. There was generally a total loss of sensations corresponding to the level of the motor lesion or with a somewhat lower cranial level. In some cases there was evidence of spasticity accompanied by ankle clonus and positive Babinski. The anocutaneous reflex was persistent in some of these cases.

*Group 2 (high lumbar)* Subtotal paraparesis was present and in some cases accompanied by residual function in the hip flexors and adductors. Loss of sensation corresponded to the level of the motor lesion but at times to a somewhat lower cranial level. In most cases reflexes were entirely absent in the lower extremities. Spasticity and/or persistent anocutaneous reflex were occasionally present.

*Group 3 (lower lumbar)* The function in hip flexors, adductors and knee extensors were well maintained. Sensation corresponded to the level of the motor lesion or to a somewhat lower cranial level. Normal or slightly decreased patellar reflexes, absent Achilles and plantar reflexes were noted. Spasticity did not occur. In some cases anocutaneous reflex persisted.

*Group 4 (high sacral)* Motor losses included impaired function of hip extensors, adductors and knee flexors as well as considerable decrease in ability to move the feet. Decreased sensation corresponding to the level of the motor lesion or to a somewhat lower cranial level was observed. Achilles and plantar reflexes were absent. Neither spasticity nor anocutaneous reflex were present.

*Group 5 (low sacral)* No motor loss was observed. Sensory loss corresponded to the lowest sacral segment. Tendon reflexes were intact. No spasticity was recorded. The anocutaneous reflex was not present.

Detailed analysis of radiological data concerning the urinary tract and correlation with the urological and neurological findings will be published separately.

## RESULTS

*Partial urinary continence* Of the 50 patients examined, 2 were completely continent and were able to control micturition. Both belonged to the upper sacral group from a neurological point of view but had unilateral functional loss. Of the remaining 48 patients four were less than 2 years of age at the time of the last examination and in 8 cases the Bricker procedure was used for urinary diversion. For this reason it is not possible to make a final judgement concerning continence in those 12 cases.

Table 1 Groups of urinary incontinence and neurological levels

	Dribbling incontinence	Dry periods of more than 1 hour's duration
Low thoracic	1	1
High lumbar	3	6
Low lumbar	0	3
High sacral	7	9
Low sacral	2	4
Total	13	23

The remaining 36 patients were divided into two groups according to the degree of continence. In the first group 13 patients had continuous dribbling incontinence or dry periods of less than 1 hour's duration. In the second group 23 patients had dry periods of at least 1 hour and often 2 to 3 hours daily. The distribution of the levels of the neurological defects appeared to be similar for both groups. None of these patients had been given bladder training (Table 1).

Eight boys and 5 girls had dribbling incontinence while 9 boys and 14 girls had partial continence for more than 1 hour.

Of those patients who later had a urinary diversion six were dribbling incontinent and one had dry periods of more than 1 hour. The eighth patient was operated on before the age of two.

Table 2 Residual bladder function and groups of incontinence

De = Detrusor function Sph = sphincter function Se = bladder sensation

Residual function	Dribbling incontinence	Dry periods of more than 1 hour	Total
De + Sph + Se	0	2	2
De + Sph	0	4	4
De + Se	0	4	4
De	1	5	6
Sph	0	0	0
Se	1	4	5
No residual function	11	2	13
No data	0	2	2
Total	13	23	36

In Table 2 both groups have been compared as regards residual function in the detrusor as well as sphincter function and bladder sensation. At least partial detrusor function was considered present when the patient was able to urinate with a stream without increasing the intra abdominal pressure. The sphincters were considered to possess some function if the bladder was not expressible and/or the patient was able to voluntarily interrupt micturition. A certain degree of retained sensation during micturition was reflected in the patient's ability to be at least partly aware of bladder filling.

In the completely dribbling incontinent group only two of 13 patients (15%) had some detrusor function or sense of bladder filling. In the group with longer dry periods corresponding values were 19 of 21 of those patients who could be evaluated (90%). Detrusor function alone or combined with other types of residual function was considerably more common in the partially continent group. Table 2 also shows that persistent sphincter function was not a necessary prerequisite for partial continence. Moreover retained bladder sensation alone was probably more common in the partially continent group. A functioning sphincter was invariably associated with signs of detrusor activity and bladder sensation. This would indicate that residual detrusor function may be the chief requirement for dry periods of more than one hour's duration. The partial continence often evolved about the age of 4 years.

*Control of defecation.* For 49 patients satis-

Table 3 *Groups of anal function and neurological levels*

	Continent	Pseudo-continent	Incontinent
Low thoracic	1	2	3
High lumbar	1	4	4
Low lumbar	1	2	2
High sacral	5	9	8
Low sacral	1	5	1
Total	9	22	18

factory data of anal function were available. The cases were divided into three groups. In the first group the patients were continent regardless of whether the stools were formed or not. In the second group (pseudo-continent) the patients were almost regularly continent if the stools were not loose. In the third group the patients were incontinent even when the stools were of normal consistency. In Table 3 the three groups are classified according to the level of the neurological lesion.

In this series it was not possible to show any definite relationship between the level of the neurological lesion and the degree of continence. More detailed analysis was made in these three groups as regards sphincter tone, anoanal reflex and sensations of ampulla filling or the need to defecate. The results are shown in Table 4. In some cases the patients were too young to be able to adequately describe ampulla sensation.

Normal sphincter tone had a favourable prognosis as had partial sphincter tone at least to some extent. In the completely incontinent group no patient had sphincter tone. The

Table 4 *Different parameters of anal function*

Degree of continence	Sphincter tone			Anoanal reflex			Ampulla sensation		
	Normal	Weak	Absent	Normal	Weak	Absent	Present	Absent	No information
Continent (9 cases)	3	6	0	3	2	4	9	0	0
Pseudocontinent (22 cases)	0	3	19	0	3	21	1	14	7
Incontinent (18 cases)	0	0	18	3	0	15	2	8	8

functions caudal to the level of the lesion. Neurological classification according to the upper level of the functional lesion resulted in the following:

**Group 1 (low thoracic)** Complete paraplegia was found sometimes accompanied by loss of strength in the trunk and particularly the abdominal muscles. There was generally a total loss of sensations corresponding to the level of the motor lesion or with a somewhat lower cranial level. In some cases there was evidence of spasticity accompanied by ankle clonus and positive Babinski. The anocutaneous reflex was persistent in some of these cases.

**Group 2 (high lumbar)** Subtotal paraparesis was present and in some cases accompanied by residual function in the hip flexors and adductors. Loss of sensation corresponded to the level of the motor lesion but at times to a somewhat lower cranial level. In most cases reflexes were entirely absent in the lower extremities. Spasticity and/or persistent anocutaneous reflex were occasionally present.

**Group 3 (lower lumbar)** The function in hip flexors, adductors and knee extensors were well maintained. Sensation corresponded to the level of the motor lesion or to a somewhat lower cranial level. Normal or slightly decreased patellar reflexes, absent Achilles and plantar reflexes were noted. Spasticity did not occur. In some cases anocutaneous reflex persisted.

**Group 4 (high sacral)** Motor losses included impaired function of hip extensors, abductors and knee flexors as well as considerable decrease in ability to move the feet. Decreased sensation corresponding to the level of the motor lesion or to a somewhat lower cranial level was observed. Achilles and plantar reflexes were absent. Neither spasticity nor anocutaneous reflex were present.

**Group 5 (low sacral)** No motor loss was observed. Sensory loss corresponded to the lowest sacral segment. Tendon reflexes were intact. No spasticity was recorded. The anocutaneous reflex was not present.

Detailed analysis of radiological data concerning the urinary tract and correlation with the urological and neurological findings will be published separately.

## RESULTS

**Partial urinary continence** Of the 50 patients examined, 2 were completely continent and were able to control micturition. Both belonged to the upper sacral group from a neurological point of view but had unilateral functional loss. Of the remaining 48 patients, four were less than 2 years of age at the time of the last examination and in 8 cases the Bricker procedure was used for urinary diversion. For this reason it is not possible to make a final judgement concerning continence in those 12 cases.

Table 1 Groups of urinary incontinence and neurological levels

	Dribbling incontinence	Dry periods of more than 1 hour's duration
Low thoracic	1	1
High lumbar	3	6
Low lumbar	■	3
High sacral	7	9
Low sacral	2	4
Total	13	23

The remaining 36 patients were divided into two groups according to the degree of continence. In the first group, 13 patients had continuous dribbling incontinence or dry periods of less than 1 hour's duration. In the second group, 23 patients had dry periods of at least 1 hour and often 2 to 3 hours daily. The distribution of the levels of the neurological defects appeared to be similar for both groups. None of these patients had been given bladder training (Table 1).

Eight boys and 5 girls had dribbling incontinence while 9 boys and 14 girls had partial continence for more than 1 hour.

Of those patients who later had a urinary diversion, six were dribbling incontinent and one had dry periods of more than 1 hour. The eighth patient was operated on before the age of two.

Table 2 Residual bladder function and groups of incontinence

De = Detrusor function Sph = sphincter function Se = bladder sensation

Residual function	Dribbling incontinence	Dry periods of more than 1 hour	Total
De + Sph + Se	0	2	2
De + Sph	0	4	4
De + Se	0	4	4
De	1	5	6
Sph	0	0	0
Se	1	4	5
No residual function	11	2	13
No data	0	2	2
Total	13	23	36

Röntgen findings were normal in all respects in four cases with fecal continence. Of those classified as pseudo-continent 2 patients showed normal findings while one demonstrated a slightly lowered pelvic floor. Both patients with incontinence (aged 1 and 9 years) had a depressed pelvic floor and were unable to retain the contrast medium injected into the rectum and to close the anal canal and the external sphincter (Fig. 1). The latter patient could not voluntarily elevate the pelvic floor.

### DISCUSSION

The incidence of urinary incontinence in unselected cases of myelomeningocele is high. Lesions located high in the cervical and upper thoracic spine often are compatible with entirely satisfactory bladder function (2, 4, 6). This is also true of hemimyelocoele (3, 7) as well as other celes in the sacral region with unilateral functional defects (5). These localisations and types however are less common. In the more common lumbosacral lesions with relatively symmetrical functional defects the incidence of incontinence is often stated to be as high as 97% (6). From a clinical point of view it is possible to distinguish between the incontinent patients who are always wet from those who have shorter or longer dry periods. In the latter group dry periods of as much as a couple of hours duration can at times be socially acceptable.

In the present series the group with dry periods of one or more hours duration is larger than the group which is permanently wet. Partial continence almost always requires some residual function of the detrusor as judged by the ability to micturate voluntarily with a stream without increasing the intra-abdominal pressure. Residual function of the sphincter was less significant, and the same applied to afferent sensation in the bladder and urethra. This type of partial and voluntarily controlled micturition is different from the trigger released reflex type of bladder-emptying prevalent in cases of high spinal-cord injury. In cases of

myelomeningocele lesions of both upper and lower motor neurons may occur. However flaccid paralysis is usually dominant and it seldom happens that enough function of the sacral cord is preserved to permit reflex emptying of the bladder. Moreover neither in this nor in other available materials was it possible to show that minor differences of the upper level of the lesion had any decisive importance for the achievement of partial continence. On the other hand partially maintained motor functions and reflexes innervated from the 2nd-5th sacral segments showed a positive correlation to residual detrusor function accompanied by partial continence (1, 3).

Smith (6) stated that the presence of large quantities of residual urine and difficulty in expressing the bladder were characteristic of subjects presenting with partial continence. The conclusion was drawn that this was related to the degree of resistance to flow in the urethra depending inter alia on sphincter function.

The present study is based on simple clinical examinations. It is likely that more detailed studies including for example simultaneous pressure-flow measurements can provide more precise information regarding bladder function and better evaluation of the prospects for successful bladder training and future continence. Using a modified method of cystometry Cooper in 1968 found normal detrusor activity in 35% of the cases studied and most of those patients were expected to become continent after bladder training. It should be noted however that 15 to 20% of the subjects showing no signs of detrusor activity became continent on training.

Eckstein (4) considered that if continence had not been achieved by the age of 3 it was unlikely to be attained later. The relatively high incidence of continent patients in his series (33%) corresponds to an unusually large number of cases with celes in the cervical or upper thoracic regions.

In clinical reports of cases of myelomeningocele comparatively little attention has been de-



*Fig 1* Male 9 years—Rectum filled with semisolid barium contrast (a and b) Marked descent of the atonic pelvic floor with caudal displacement of the rectum partial obliteration of the wedge of the levator ani muscle immediately posterior to the rectum comprising the puborectalis component and funneling of the anal canal which is incompletely closed (c) The cooperative patient is unable to elevate the pelvic floor



sensation of fullness of the ampulla or the desire to defecate showed a strong positive correlation to the subsequent development of continence although exceptions occurred. All patients who had both ampulla sensation and some degree of sphincter tone were completely continent. The anocutaneous reflex showed a poor correlation to the degree of continence.

It should be noted that patients who developed a satisfactory degree of anal continence did so when relatively young, many at 2–3 years of age. In the second group pseudocontinence developed around 7 years of age. Several of the completely incontinent patients were quite young and classified in group 3 for the present.

## RESPIRATORY PATHOLOGY IN THE IMMEDIATE POSTNATAL PERIOD

A. BALLABRIGA A. MORAGAS A. GALLART-CATALA and N. BARAT

*From the Children's Hospital of the "Seguridad Social"  
(Head A. Ballabriga) Barcelona, Spain*

Respiratory disorders in the immediate postnatal period correspond to a clinical syndrome which represents diverse anatomical disorders of the lung the majority of which may only be differentiated and classified through a very close histological examination. It is not possible to establish a strict correlation between the clinical symptoms and the pulmonary anatomical findings.

Our work is mainly orientated on the incidence of severe forms of respiratory distress classified according to the Silverman Andersen retraction score. Relationship with findings in a large series of post mortem examinations have been obtained in order to establish the frequency of pulmonary involvement according to age and weight.

### MATERIAL AND METHODS

The entire tabulated material has been personally observed in the Children's Hospital and in the attached Maternity unit during a 27 months period (March 1966 to May 1968).

In a total of 32 953 observed infants 31 570 were full terms and 1 433 premature. In the full term group 31 330 were born in the Maternity Hospital and 190 in different clinics of the city and admitted afterwards to our Unit for Pathological New borns.

In the group of 1 433 premature 933 were born in the Maternity Hospital and 500 were admitted to our Unit for Prematures during the first day of life proceeding from local clinics.

Apgar index taken 1 min after birth was studied in 20 000 full term infants proceeding from the group

of 31 330 born in the Maternity Hospital and also in the group of 933 premature. Silverman & Andersen's (14) retraction score at 5 hours of life was studied in all 32 953 cases.

For the estimation of incidence of respiratory distress we have included all cases showing a retraction score in stages I or II at 5 hours of life. Cases in stages 0 at the moment of the first examination but showing during the following 72 hours a clinical evolution to stages I or II were also tabulated.

Variations of retraction score after 72 hours of life were not considered in our statistics because acquired infections after birth may play a role in these cases.

Microscopic examination was performed in all or parts with special emphasis to the lungs.

The pathological material has been studied by the same team according to a previously established criteria however if there is a certain personal influence in the selection—as happens in all anatomicopathological classifications—this is extended uniformly over all the material. The lung was classified according to the most extensive and important histological features that could condition the death. The material was divided under the following headings:

(a) Hyaline membranes the undoubted typical forms are included. When material was observed only suggesting the diagnosis of "membrane" the case was classified according to the other existing lesions.

(b) Atelectasis without hyaline membranes. These cases have been included because at times they are the only pulmonary alterations present, without being considered as the primary cause of death. Occasionally the existence of another acute extrapulmonary cause was quoted.

(c) Immaturity this has been classified in 4 grades. Grade III corresponds to the maximum degree of immaturity. It refers to the lung in canalicular state with the presence of cartilage and thick septa with collagenized stromal tissue and the capillary vessels removed from the alveolar air spaces. Stages I

voted to the problem of anal continence. It is generally stated that in most cases a socially acceptable continence exists, provided the patient is kept constipated. Here, of course, diet and a strict defecation regime may play an important role. The extent of paresis of the extremities may indirectly affect the prospects of success for such a programme. Detailed analysis of the neurological conditions favouring anal continence have not been published. In the present material, as in earlier published studies, evidence of anal sphincter function was more common when the cele was located in the lower part of the thoracic cord or the upper part of the lumbar cord. In our material, the patient's ability to experience the fullness of the ampulla and an urge to defecation was of great significance. These afferent functions appear to have no direct relationship to the level of the lesion of the cele. They were to a large extent positively correlated to complete anal continence, regardless of the consistency of stools.

It is apparent that the present knowledge of the factors of significance for bladder and anal continence are only incompletely understood and that further detailed clinical and roentgenologic studies of cases of myelomeningoceles might be of help in designing further measures to promote continence.

### SUMMARY

Patients with myelomeningocele are due to present with varying degrees of urinary and anal incontinence. The present study represents an attempt to elucidate the factors of signifi-

cance for selection of patients suitable for training to achieve socially acceptable continence. It would seem that an active detrusor is the chief factor determining urinary continence, whereas sphincter function and bladder sensation appear less significant. As regards anal continence, sensation in the ampulla seems most important.

### REFERENCES

- 1 Cooper D G. Bladder studies in children with neurogenic incontinence. With comments on the place of pelvic floor stimulation. *Brit J Urol* 40: 157 1968.
- 2 Doran P A & Guthkelch A N. Studies in spina bifida. Part IV. The frequency and extent of paralysis. *J Neurol Neurosurg Psychiat* 26: 545 1963.
- 3 Duckworth T, Shurrard W J, Lister J & Seymour N. Hemimyelomeningocele. *Develop Med Child Neurol Suppl* 16: 69 1968.
- 4 Eckstein H B. Urinary control in children with myelomeningocele. *Brit J Urol* 40: 191 1968.
- 5 Ericsson N O, Hellström B, Nergårdh A & Rudhe U. Unilateral neurological defect in myelomeningocele with normal bladder function. *Acta Paediat Scand* 59: 487 1970.
- 6 Smith E D. *Spina bifida and the total care of spinal myelomeningocele*. Charles C Thomas, Springfield, Ill 1965.
- 7 Stark G. Pathophysiology of the bladder in myelomeningocele and its correlation with the neurological picture. *Develop Med Child Neurol Suppl* 16: 76 1968.

Submitted Dec 8 1969

Accepted Jan 8 1970

(N O E) Dept. of Pediatrics  
Karolinska sjukhuset  
104 01 Stockholm 60  
Sweden

**Key words:** Myelomeningocele, urinary continence, anal continence.

Table 3 Distribution of 700 autopsic cases according to age

	Period					Total
	0-24 h	24-48 h	48 h-5 days	5-10 days	10-30 days	
Pretermes 500-1 250 g	66	24	26	11	5	132
Pretermes 1 251-2 500 g	106	52	59	39	51	307
Full term newborns > than 2 500 g	81	47	62	24	47	261
						700

FT - Full term P - Prematures

Statistical treatment of data of this group and the main pathological findings were made with the following formula

$$Qr = \frac{a1+a2}{+} \quad S^2d = Qr(1-Qr) \left( \frac{1}{a1} + \frac{1}{a2} \right)$$

$$r = \frac{q1-q2}{sd}$$

## RESULTS

**A Study of the Apgar index** Results concerning 1 min Apgar index (2-3) performed in 20 000 full terms and 933 prematures have been compared with the results obtained in various previous studies and the distribution of neonatal mortality classified by scores. The

mortality rate is related to the period 0-30 days of life and the frequency and mortality of each score is quoted in Table 1

**B Study of the frequency of respiratory distress in full term and premature infants** The study of the frequency of the appearance of respiratory distress has been based on a batch of 31 330 observed full term births (normal vaginal delivery 89.4%—forceps 3.09%—Caesarian section 2.9%—bridge delivery 2.6%—vacuum 1.6%—Version 0.1%) classified according to the Silverman score and the results were as follows. Full term births observed 31 330—number of respiratory distress

Table 4 Distribution of pulmonary findings in 700 infants (439 prematures and 261 full terms) dead in the period 0-30 days of life

	0-24 h		24-48 h		48 h-5 days		Total 0-5 days		5-10 days		10-30 days		Total 0-30 days	
	FT	P	FT	P	FT	P	FT	P	FT	P	FT	P	FT	P
Total number	81	172	47	76	62	85	271*	475*	24	50	47	56	101	151
Hyaline membrane	12	72	12	37	8	16	170	374	0	2	0	0	0	18
Atelectasis without H.M. <sup>a</sup>	13	16	6	5	7	7	136	84	0	4	1	1	14	47
Immaturity <sup>d</sup>	0	30	0	9	0	8	0	141	0	2	0	0	0	18
Aspiration	8	4	6	3	6	6	105	39	2	2	0	2	28	37
Haemorrhages <sup>d</sup>	15	13	4	7	12	17	163	111	7	12	2	7	126	179
Pulmonary hypoplasia	13	8	0	0	0	0	68	24	1	0	0	0	14	0
Pneumonia	12	18	8	9	18	28	200	165	9	27	31	33	560	584
Listeriosis	1	6	1	1	1	1	15	24	0	0	1	0	14	0
Absence of pulmonary participation	7	5	10	5	10	2	142	36	5	1	12	11	240	113

\* The number of severe asphyxiated cases in the whole of this series was 26/190-13.6%.

<sup>a</sup> The number of severe asphyxiated cases in the whole of the series of prematures was 36/333-10.8%.

<sup>b</sup> The number of gross congenital abnormalities in the whole of these series was 13/106-12.2% and 6/71-8.4% respectively.

<sup>d</sup> In 31 prematures belonging to these three groups brain damage was demonstrated which could be the main cause of death and in 70 cases of the full term series.



Table 1 *Distribution of neonatal mortality by scores in various studies*

Author	Total no of infants	Total mortality ( )	Total full term	Total prematures	Apgar index		
					Score 10-7 ( )	Score 6-4 ( )	Score 3-1 ( )
Apgar & James 1962	27 715	17	—	—	F 79.3 M 0.4	F 13.3 M 2.2	F 7.4 M 2.1
Drage et al 1964	17 221	14	—	—	F 78.9 M 0.4	F 14.5 M 2.1	F 6.1 M 1.1
Ikonen 1967	11 855	12	—	—	F 96.9 M 0.5	F 1.9 M 13.2	F 1.2 M 34.1
This study	20 933	14	—	20 000	F 95.5	F 2.89	F 1.5
					M 0.6	M 12.6	77 28.4
					F 96.4	F 2.39	F 1.1
					M 0.15	M 7.7	M 19.2
					F 76.8 M 13.3	F 13.6 M 33.5	F 9.4 M 53.4

F = Frequency M = Mortality

shows zones with discreet signs of immaturity slight alveolar development and cuboid type epithelial alveolar investment. Grade II represents the intermediate forms between Grades III and I. Grade 0 is the normal mature lung.

(d) Aspiration. In the 0-5 days group only the massive forms of amniotic or meconial aspiration have been considered. The 5-30 day group includes fundamentally alimentary aspiration at times with pulmonary granulomatous reaction and aspiration of gastric juices frequently associated with a necrotizing pneumonia.

(e) Haemorrhages. The massive forms of haemorrhage have been included which affect uniformly two or more complete lobes and large diffuse intra-alveolar and interstitial submassive haemorrhages considering the extrusion of pulmonary fragments from different zones.

(f) Pulmonary hypoplasia. These represent the cases which presented a total pulmonary mass weighing less than 50% of the normal weight for the age and body weight independently of the degree of pulmonary maturity. All the cases included in this group were associated with severe malformations as diaphragmatic hernia and/or cystic renal dysplasia or renal agenesis.

(g) Pneumonia. In the group of deaths occurring from 0-48 hours there are included the forms of intrauterine pneumonia with very abundant infiltration of leukocytes without the presence of fibrine

without necrosis and with or without pathogenic bacteria. Beyond 48 hours all the types of pneumonia are catalogued as acquired pneumonias. Included in this group of acquired pneumonias are (a) necrosis producing or haemorrhagic granulocytary pneumonia (b) interstitial pneumonia (c) interstitial with giant cells (d) pneumonia accompanying aspiration with various degrees of desquamative alveolitis (e) pneumonia with presence of candida albicans.

(h) Listeriaemia. There are included the pulmonary forms with disseminated pulmonary granulomatosis and identification of listeria in the lung tissue.

(i) Absence of pulmonary participation. These include the cases in which there were no osteoblastic findings in the lung and the main cause of death was removed from the lung. Nevertheless it is frequently possible to find included in this group a minimum pulmonary histological participation in the form of a minimum granulocytary exudation reduced to the presence of 3 to 4 leukocytes per alveolar air space without uniform distribution. Although a very high number of these deaths had presented asphyxia at the time of birth we have classified the unova isolated among the pulmonary findings and these cases are included within the group of the respective alterations they presented.

Incidence of respiratory distress in 406 sets of twins was also studied. These twins were taken in the group of infants born in the Maternity Hospital.

Table 2 *Incidence and mortality in severe respiratory distress in prematures*

	Total numbers	Very severe distress	Frequency ( )	Death cases	Mortality ( )
Prematures 500-1 250 g	112	40	35.7	38	95
Prematures 1 251-2 500 g	1 321	49	3.7	44	89.9

respiratory distress in a total of 31 330 children born in the Maternity Hospital 16 313 were males and 15 017 females giving a ratio of 1 male to 0.920 female. The number of respiratory distress cases was 742 of which 432 males and 310 females giving a ratio of 1 male/0.717 female. Thus the incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female births the difference being significant.

C Incidence, severity and mortality rate of respiratory distress in twins with reference to sex. Muir & Futrakul (13) have recently reported a higher incidence of respiratory distress in males over females especially in twins of opposite sexes. The clinical course and severity of the respiratory distress was the same in both sexes in their series. Our data is composed of a total of 406 sets of twins of which 153 sets were boys, 126 sets were girls and 127 pairs were boy and girl (total 433 males and 379 females). This group has taken into account only those cases which presented a severe form of respiratory distress (Stadium II of Sherrman) and cyanosis requiring the administration of oxygen.

Severe respiratory distress was present in 9.93% of males and 8.7% of females. This slightly higher incidence in males studied by the homogeneous test between two averages was not significant ( $p$  between 0.6 and 0.5). The number of deaths in both sexes considering the mortality during the first 10 days of life in both sexes has been similar (6.9% and 7.1%). The most important anatomical findings in these cases (autopsy index 91.2%) were similar in both sexes and correspond to typical forms of asphyxia with hyaline membranes (66.6% and 68%) respectively. Pulmonary immaturity and pulmonary haemorrhages were the most frequent accompanying findings.

III Macro- and microscopic post mortem examination on 700 cases. The series of 700 post mortem examinations performed on cases of children deceased during the period 0-30 days

Table 7 Relation between sex and pulmonary maturity

Pulmonary maturation	Total cases	Males	Females
Type III	40	18	22
Type II	55	29	26
Type I	31	16	15
Type 0	6	3	3
Total	132	66	66

of life has been classified into 3 groups corresponding to premature birth from 500 to 1 250 g, premature births from 1 251 to 2 500 g and full term births with weight in excess of 2 500 g. The distribution of this series is quoted in Table 3.

The classification of pulmonary findings in the post mortem examinations of 439 premature and 261 full term deaths in the period 0-30 days of life is shown in Table 4.

The incidence of the hyaline membrane according to weight groups showed a notable greater frequency in the 1 250 to 2 500 g weight group. This incidence taking the 0-5 days death group and a comparison with different statistics from the literature is quoted in Table 5.

In the 160 cases of hyaline membrane studied with a typical histological finding the associated pulmonary histological lesions apart from asphyxia and immaturity to a variable extent were as follows: discrete pulmonary haemorrhages 23.7%, minimum granulocytary exudation 16.2%, acquired pneumonia 7.5%, intralobular pneumonia 3.1%, massive pulmonary haemorrhage 2.5%, asphyctic syndrome 2.5%, infectious 0.6%, brain lesions comprising subarachnoid haemorrhages 20.6%.

With reference to the type of birth the greatest incidence of hyaline membrane occurred in premature and in full term delivered by caesarean section or breech delivery with a frequency rate 3 to 6 times greater.

In the 160 cases of hyaline membrane found in our series of 700 autopsies 10.6% were

Table 5 Autopsic incidence of hyaline membrane according to weight groups compared with various previous studies

Author	Period	Weight	Age	Autopsies	H M	H M %
Swanston	59/60	500-2 500	1 week	304	116	38.1
Latham et al	37/49	1 000-2 500	1 week	257	98	38.1
Avery & Oppenheimer	44/48	1 000-2 500	30 min-6 days	70	17	24.3
Avery & Oppenheimer	54/58	1 000-2 500	30 min-6 days	145	56	38.6
Ahvenainen & Terhin	54/61	600-2 500	—	174	40	23.5
Hirvonsalo & Arko	61/65	600-2 500	1 week	319	68	21.3
This study	66/68	500-2 500	0-5 days	333	126	37.8
This study	66/68	500-1 250	0-5 days	116	20	17.2
This study	66/68	1 251-2 500	0-5 days	217	106	48.8
Hirvonsalo & Arko	61/65	+ 2 500	1 week	120	15	12.5
This study	66/68	+ 2 500	0-5 days	190	32	16.8

in stadia I or II 2 036—overall frequency 6.4\*

In prematures the frequency is much greater and also depends on the weight

The incidence was much higher in the low birth weight group and in this way in 112 cases of weight ranging between 500 and 1 250 the frequency of distress was about 90.1%, in front of a frequency of 23.9% in the group of 1 321 prematures with weight ranging between 1 251 and 2,500 g.

Miller sets frequencies of 81.8% and 24.4% respectively for the groups with weight ranging between 1 000-1 500 and 2 001-2,500 g. The total frequency of our series of respiratory distress (moderate and severe cases) over a total of 1 433 prematures weighing 500-2,500 g = 29.03.

The incidence of severe forms in Silverman stadium II with cyanosis requiring permanent administration of oxygen is quoted in Table 2. Post mortem examinations were performed on

all the 38 prematures weighing less than 1,250 g who died of severe respiratory distress and in the group of 44 weighing more than 1,250 g who died, post mortem examinations were performed in all except one. In the group of prematures of 500-1,250 deceased during the period of 0-10 days of life, as a result of severe clinical respiratory distress and who post mortem examination the predominant alteration was pulmonary immaturity with hyaline membrane in 10.5% of the cases pulmonary haemorrhage in 10.5% and pneumonia in 26.3%. In the group of 43 cases with weight ranging between 1,251 and 2 500 g death in the same period of life the main pulmonary finding was hyaline membrane with an incidence of 39.5% pulmonary haemorrhage was present in 20.4% of the cases and pneumonia only in 4.5% of them.—In this latter group a 25.5% incidence of severely malformed infants was encountered.

With reference to the incidence by sex of

Table 6 Incidence of pulmonary haemorrhages

Author	Period	Weight	Age	No of autopsies	Pulm haemorrh	Frequency in
Esterly & Oppenheimer	1954/64	0-2 500	2 weeks	668	94	14
Esterly & Oppenheimer	1954/65	+ 2 500	2 weeks	90	41	45.6
Ballabriga & Moragas	1966/68	500-2 500	0-10 days	383 <sup>a</sup>	49	12.7
Ballabriga & Moragas	1966/68	+ 2 500	0-10 days	214 <sup>a</sup>	38	17.7

\* The cases of hyaline membrane and pulmonary haemorrhage have been tabulated only under the heading of hyaline membrane.

<sup>a</sup> This group contained 21.9% of pneumonias (47/214). A large number of them offered extensive pulmonary haemorrhages but these cases have been catalogued as pneumonias.

total period varies according to different authors. This depends on the strictness of the anatomical and clinical classification adopted. Knecht (9) found massive intraalveolar haemorrhages in 4.2% of the live birth autopsy findings of newborns up to one month of age. In 336 neonatal deaths studied by Thorburn (16) 8% pulmonary haemorrhages were found.

Our data concerning pulmonary haemorrhages in prematures show an incidence of 17.7% as the cause of death similar to the data of Esterly & Oppenheimer (6) of 14

### CONCLUSIONS

The incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female births; the difference being significant.

The high incidence of hyaline membrane has been found in the group ranging in weight between 1 251-2 500 g (48.8% of 217 autopsies).

The incidence of intrauterine pneumonia has been higher in the group with weight ranging between 500-1 250 g and death during the first day of life.

The frequency of hyaline membrane with reference to the type of delivery is higher in groups born by caesarean section or breech delivery.

In the groups of deaths between 5-30 days of life pneumonia has been the main cause of death in full terms as well as in prematures.

### SUMMARY

The study of one minute Apgar index in 20 000 full term babies and 931 prematures showed a frequency of the score 0-3 of 1.9% in the group of full term babies and 9.4% in the group of prematures. The neonatal mortality in this material was 1.4.

The incidence of respiratory distress in 31 350 full term babies classified according to the Sierstman & Andersen score was 6.4%.

In a group of 112 prematures with weight

ranging between 500-1 250 g the incidence of respiratory distress was 90.1% and in 1 321 prematures with weight ranging between 1 251-2 500 g was 23.9%.

In relationship with the incidence of respiratory distress in twins the study of 406 sets of twins shows that it has not been possible to observe a predominance of severe forms in either sex likewise the number of deaths in both sexes has been similar considering the mortality during the first 10 days of life.

Histological examination in 700 autopsies (deaths in the period 0-30 days of life) showed that in a group of 333 prematures with deaths in the period 0-5 days of life the incidence of hyaline membrane was 37.4% and in a group of 190 full terms autopsied in the same period of life was 17% this difference being significant.

The frequency of hyaline membrane with reference to the type of delivery shows a high incidence in groups born by caesarean section or breech delivery. The incidence of massive pulmonary haemorrhages was 12.7% among 383 autopsies of prematures and 17.7% among 214 full term babies autopsied during the period 0-10 days of life.

Incidence of lacerations as main cause of death in 700 post mortem examinations performed during the period 0-30 days of life was 1.71%.

### REFERENCES

1. Abrahamson E. K. & Terbo A. Mortality of low birth weight in infants in a General Hospital. *Ann Perinat Fetus* 14 11 1968.
2. Apgar V. The newborn (Apgar) scoring system. Reflection and advice. *Pediatr Clin N Amer* 13 465 1966.
3. Apgar V. & James L. B. Further observations on the newborn scoring system. *Amer J Dis Child* 108 419 1962.
4. Avery M. E. & Oppenheimer E. H. Recent increase in mortality from hyaline membrane disease. *J Pediatr* 57 553 1960.
5. Drage J. B. Kennedy C. & Schwartz B. A. The apgar score as an index of neonatal mortality. *Obstet Gynec* 24 222 1964.
6. Esterly J. R. & Oppenheimer E. H. Massive pulmonary haemorrhage in the newborn. I. Pathologic considerations. *J Pediatr* 69 3 1966.

born by caesarean section and 17.5% by breech delivery meanwhile the usual percentage of caesarean section and breech delivery in the total deliveries of our Maternity Hospital amounted 2.9% and 2.6% respectively.

The incidence of pulmonary haemorrhages in the post mortem examinations in our series have been compared with the series of Esterly & Oppenheimer (6) and results have been quoted in Table 6.

The relation between sex and pulmonary maturity has been examined in 132 lungs from prematures ranging weight between 500 and 1,250 g and the results are quoted in Table 7.

The sex ratio in all the cases of respiratory distress observed has been 1 male/0.717 female and in pulmonary immaturity the ratio has been 1 male/1 female.

## DISCUSSION

In relationship to the data obtained with the one minute Apgar score (2-3) in our series and in comparison with various previous studies, it is seen that there is a greater number of asphyctic children at the time of birth in the two sets of American statistics than in the European ones, but a greater number of them may be recovered with a lower mortality rate. This may be because in the European series the administration of analgesics and sedatives is perhaps less and therefore the cases with low scores in our series correspond to very severe forms with a high mortality rate.

On the other hand in the series of premature infants with scores between 7 and 10 the mortality rate is notably higher if compared with the full terms offering the same score and this must be considered from the point of view the postnatal mortality of prematures during the first month of life is influenced by other factors. The prognosis with reference to mortality established by the Apgar index is better for full term infants than premature ones.

Concerning pulmonary findings as cause of death the lung has been classified according to the most important and extensive feature but

very frequently this primary cause is mixed and there are several factors which can contribute as important associated causes. The incidence of the hyaline membrane according to weight groups showed a notable greater frequency in the 1,250 to 2,500 g group.

The association between hyaline membrane and intrauterine pneumonia has been an exception in our series, on the other hand the association between fragmented hyaline membranes in period of reabsorption and acquired pneumonia have been frequent in the group of deaths occurring between 48 hours and 10 days of life.

Pulmonary immaturity has been frequently associated with atelectasis and interstitial emphysema, likewise there is a frequent association between hyaline membrane atelectasis, interstitial emphysema and immaturity of variable degree.

The frequency of hyaline membrane in the series of prematures dying in the period 0-5 days of life (37.53%) compared to the frequency in full terms dead in the same period (16.8%) was significantly ( $p < 0.001$ ) higher.

The difference in frequency of pulmonary haemorrhages and pneumonias in the period 0-5 days of life between prematures and full terms was not significant ( $p > 0.05$ ). Neither exists a significant difference ( $p > 0.05$ ) between the percentage of pneumonia in the period 5-30 days of life in prematures (58.49%) and full terms (56.33%).

Pulmonary immaturity in its two advanced grades types II and III was frequently associated with intrauterine pneumonia while the hyaline membrane is more commonly associated with the groups grade 0 and I of pulmonary maturity.

The incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female birth and confirming Linding's (10) findings that pulmonary immaturity shows a slight predominance in the female sex.

The incidence of pulmonary haemorrhages in the post mortem materials from the post

natal period varies according to different authors. This depends on the strictness of the anatomical and clinical classification adopted. Knecht (9) found massive intrapulmonary haemorrhages in 4.2% of the live birth autopsy findings of newborns up to one month of age. In 336 neonatal deaths studied by Thorburn (16) 8% pulmonary haemorrhages were found.

Our data concerning pulmonary haemorrhages in premature show an incidence of 17.7% as the cause of death similar to the data of Esterly & Oppenheimer (6) of 14%.

### CONCLUSIONS

The incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female births the difference being significant.

The highest incidence of hyaline membrane has been found in the group ranging in weight between 1 251-2 500 g (48.8% of 217 autopsies).

The incidence of intrauterine pneumonia has been higher in the group with weight ranging between 500-1 250 g and death during the first day of life.

The frequency of hyaline membrane with reference to the type of delivery is higher in groups born by caesarean section or breech delivery.

In the groups of deaths between 5-30 days of life pneumonia has been the main cause of death in full terms as well as in premature.

### SUMMARY

The study of one minute Apgar index in 70 000 full term babies and 933 premature showed a frequency of the score 0-3 of 1.9% in the group of full term babies and 9.4% in the group of premature. The neonatal mortality of this material was 1.4%.

The incidence of respiratory distress in 31 330 full term babies classified according to the Sierman & Andersen score was 6.4%.

In a group of 112 premature with weight

ranging between 500-1 250 g the incidence of respiratory distress was 90.1% and in 1 321 premature with weight ranging between 1 251-2 500 g was 23.9%.

In relationship with the incidence of respiratory distress in twins the study of 406 sets of twins shows that it has not been possible to observe a predominance of severe forms in either sex likewise the number of deaths in both sexes has been similar considering the mortality during the first 10 days of life.

Histological examination in 700 autopsies (deaths in the period 0-30 days of life) showed that in a group of 333 premature with deaths in the period 0-5 days of life, the incidence of hyaline membrane was 37.4% and in a group of 190 full term autopsied in the same period of life was 17% this difference being significant.

The frequency of hyaline membrane with reference to the type of delivery shows a high incidence in groups born by caesarean section or breech delivery. The incidence of massive pulmonary haemorrhages was 12.7% among 383 autopsies of premature and 17.7% among 214 full term babies autopsied during the period 0-10 days of life.

Incidence of pneumonia as main cause of death in 700 post mortem examinations performed during the period 0-30 days of life was 1.71%.

### REFERENCES

1. Ahlstrom E. K. & Terho A. Mortality of low birth weight in infants in a General Hospital. *Ann Paediatr Fenniae* 14 11 1968.
2. Apgar V. The newborn (Apgar) scoring system. Reflection and advice. *Pediatr Clin N Amer* 13 643 1966.
3. Apgar V & James L. S. Further observations on the newborn scoring system. *Amer J Dis Child* 104 419 1962.
4. Avery M. E. & Oppenheimer E. H. Recent increase in mortality from hyaline membrane disease. *J Pediatr* 57 333 1960.
5. Drago I. S., Kennedy C. & Schwartz B. K. The Apgar score as an index of neonatal mortality. *Gynec Obstet* 24 222 1964.
6. Esterly S. K. & Oppenheimer E. H. Massive pulmonary haemorrhage in the newborn. I. Pathologic considerations. *J Pediatr* 69 3 1966.

born by caesarean section and 17.5% by breech delivery meanwhile the usual percentage of caesarean section and breech delivery in the total deliveries of our Maternity Hospital amounted 2.9% and 2.6% respectively.

The incidence of pulmonary haemorrhages in the post mortem examinations in our series have been compared with the series of Esterly & Oppenheimer (6) and results have been quoted in Table 6.

The relation between sex and pulmonary maturity has been examined in 132 lungs from prematures ranging weight between 500 and 1250 g and the results are quoted in Table 7.

The sex ratio in all the cases of respiratory distress observed has been 1 male/0.717 female and in pulmonary immaturity the ratio has been 1 male/1 female.

## DISCUSSION

In relationship to the data obtained with the one minute Apgar score (2-3) in our series and in comparison with various previous studies, it is seen that there is a greater number of asphyctic children at the time of birth in the two sets of American statistics than in the European ones but a greater number of them may be recovered with a lower mortality rate. This may be because in the European series the administration of analgesics and sedatives is perhaps less and therefore, the cases with low scores in our series correspond to very severe forms with a high mortality rate.

On the other hand in the series of premature infants with scores between 7 and 10 the mortality rate is notably higher if compared with the full terms offering the same score and this must be considered from the point of view the postnatal mortality of prematures during the first month of life is influenced by other factors. The prognosis with reference to mortality established by the Apgar index is better for full term infants than premature ones.

Concerning pulmonary findings as cause of death the lung has been classified according to the most important and extensive feature but

very frequently this primary cause is mixed and there are several factors which can contribute as important associated causes. The incidence of the hyaline membrane, according to weight groups showed a notable greater frequency in the 1,250 to 2,500 g group.

The association between hyaline membrane and intrauterine pneumonia has been an exception in our series, on the other hand the association between fragmented hyaline membranes in period of reabsorption and acquired pneumonia have been frequent in the group of deaths occurring between 48 hours and 10 days of life.

Pulmonary immaturity has been frequently associated with atelectasis and interstitial emphysema, likewise there is a frequent association between hyaline membrane, atelectasis, interstitial emphysema and immaturity of variable degree.

The frequency of hyaline membrane in the series of prematures dying in the period 0-5 days of life (37.53%) compared to the frequency in full terms dead in the same period (16.8%) was significantly ( $p < 0.001$ ) higher.

The difference in frequency of pulmonary haemorrhages and pneumonias in the period 0-5 days of life between prematures and full terms was not significant ( $p > 0.05$ ). Neither exists a significant difference ( $p > 0.05$ ) between the percentage of pneumonia in the period 5-30 days of life in prematures (58.49%) and full terms (56.33%).

Pulmonary immaturity in its two advanced grades types II and III was frequently associated with intrauterine pneumonia while the hyaline membrane is more commonly associated with the groups grade 0 and I of pulmonary maturity.

The incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female birth and confirming Landing's (10) findings that pulmonary immaturity shows a slight predominance in the female sex.

The incidence of pulmonary haemorrhages in the post mortem materials from the post

## THE FATTY ACID COMPOSITION OF DEPOT FAT IN CHILDHOOD

### 1 The effect of age sex and site in superficial fat

JOHN A BIRKBECK

From the Department of Paediatrics University of British Columbia

The fatty acid composition of the storage triglycerides of human depot fat has been reported by several workers particularly since the introduction of the precise analytical technique of gas liquid chromatography (19). Some authors (e.g. 31) have regarded human depot fat as relatively uniform in fatty acid composition although there is much evidence to the contrary.

In the adult, there is little evidence that in health the pattern changes with age. However several studies of the newborn period have demonstrated major differences from the adult pattern. Such differences are commonly ascribed to the effect of different dietary lipid composition although they are demonstrable prior to feeding at birth and vary between full term and premature infants.

Studies of subjects from birth to maturity are scanty. The present report deals with an investigation into the influence of age sex and sampling site on the fatty acid pattern of subcutaneous depot fat in children.

## METHODS

The study population consisted of individuals between birth and maturity admitted to a Children's Hospital for minor elective surgical procedures. Most of these were hernia repairs, minor orthopaedic manipulations and plastic repairs. All subjects were judged by examination to be otherwise healthy and free of any evidence or family history of metabolic disorder. Although long term dietary habits do influence depot fat composition it was not deemed practicable to

undertake retrospective dietary assessments on the subjects. So far as could be determined all were eating "typical" mixed North American diets.

It was felt that the occasional individual with minor peculiarities of diet habit would affect the overall results very little due to the large sample size. Most of the subjects were of Caucasian origin, the few Orientals were on a North American type of diet. There were 48 satisfactory analyses of tissue from the anterior abdominal wall (38 male and 10 female) and 35 samples from limb sites (8 male and 27 female).

Fat samples typically 0.1 to 0.5 g in weight from accessible sites at surgery were removed cleanly by the surgeon at the start of the operation. The sample was immediately placed in a tube with sterile saline containing hydroquinone as an antioxidant and frozen as soon as practicable. A preliminary study failed to reveal any effect of the storage procedure, the hydroquinone or the duration of storage on the analytical results.

A small (30-50 mg) sample of the tissue was minced. The lipids were hydrolysed and the fatty acids converted to their methyl esters by the method of Metcalfe *et al.* (29) as modified by Rodriez (6). The hydrolyses and esterification were always carried out immediately prior to chromatography as it was found that storing the esters at room temperature gave unreliable results. In this way close replication was achieved.

The ester mixture was analysed on a P & M model 810 dual column gas chromatograph using hydrogen flame ionisation detectors. The columns were 10-EGSS-X on a support of Gas-Chrom P (100-120 mesh) the aluminium column being 8 feet in length and 1/8 inch o.d. The carrier gas was helium. Temperature programming between 150 and 200°C was employed.

The peak areas were quantitated with a Desk integrator and the results expressed on the basis of relative molar response as a percentage of the total (36). That is the quantitation was corrected for small differences in detection response to acids of differing



- 7 Hirvensalo, M. & Arko, H. Studies in perinatal mortality in a large Maternity Hospital. *Ann Paediat Fenniae* 13: 46, 1967.
- 8 Ikonen, R. S. The Apgar scoring of newborn infants and its relation to neonatal mortality. *Ann Paediat Fenniae* 13: 111, 1967.
- 9 Knecht, U. Die intraalveolare Massenblutung des Neugeborenen. *Helvet Paediat Acta* 21: 268, 1966.
- 10 Landing, B. H. Pulmonary lesions of newborn infants. A statistical study. *Pediatrics* 19: 217, 1957.
- 11 Latham, E. F., Nesbitt, E. E. L. & Anderson, O. W. A clinical pathological study of newborn lung with hyaline like membranes. *Bull Hopkins Hosp* 96: 173, 1955.
- 12 Miller, H. C. Respiratory distress syndrome of newborn infants. I. Diagnosis and incidence. *J Pediatr* 61: 2, 1962.
- 13 Miller, H. C. & Futrakul, P. Birth weight, gestational age and sex as determining factors in the incidence of respiratory distress syndrome of prematurely born infants. *J Pediatr* 72: 628, 1968.
- 14 Silverman, W. A. & Andersen, E. H. A controlled clinical trial of effects of water mist on obstructive respiratory signs, death rate and autopsy findings among premature infants. *Pediatrics* 17: 1, 1956.
- 15 Sivaneasan, E. Neonatal pulmonary pathology. Singapore. *J Pediatr* 59: 600, 1961.
- 16 Thorburn, M. L. Neonatal death and mass pulmonary haemorrhage in Jamaica. *Arch Childh* 38: 589, 1963.

Submitted Jan. 30, 1969

Accepted July 28, 1969

Children's Hospital of the "Seguridad Social"  
Paseo Valle Hebrón  
Barcelona  
Spain

**Key words:** Incidence, respiratory distress, hyaline membrane, pulmonary haemorrhage, mortality.

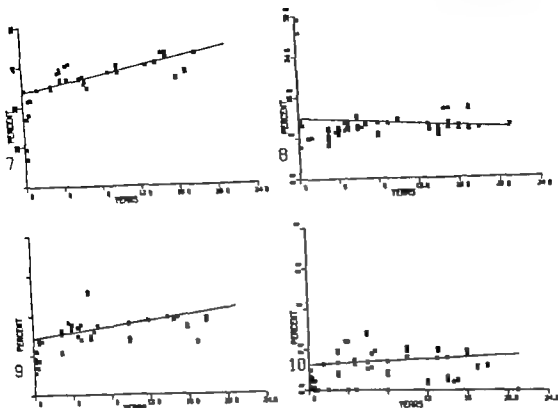


Fig 1 Relationship of fatty acid composition with age  
Ordway's fatty acid as percent of total abdominal age

in years Graph 1 = 120 2 140 3 160 4 161  
5 170 6 180 7 181 8 182 9 183 10 204

decanoic acid (17:0) was used as an internal standard but with the routine use of standard mixture (the NIH standards plus Standard No 16) for calibration the instrument was found to be sufficiently stable that retention time alone was satisfactory for identification. For a more detailed analysis of the minor and trace components of human depot fat the reader is referred to the monograph studies of Kneibury and colleagues (21, 22).

## RESULTS

The analyses of 83 samples of subcutaneous fat were reviewed. Since it was possible that age, sex and site of sampling might be relevant variables the data was subjected to computer analysis.

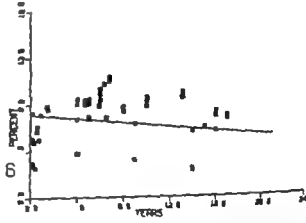
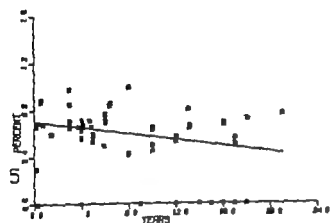
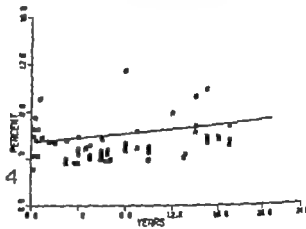
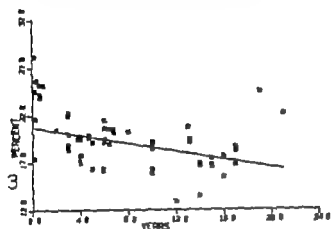
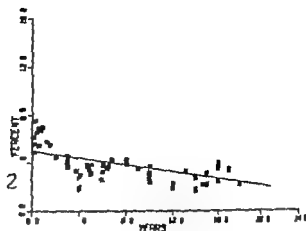
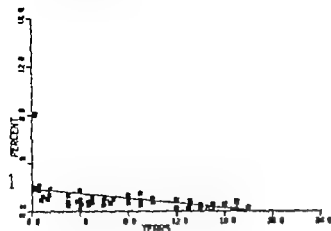
The reproducibility of the method was very satisfactory. Every sample was analysed at least twice and most often three times. Replication was within the limits found for the NIH standards in fact within 1% for major peaks and 2.5% for others.

The fatty acids identified were 10:0, 12:0, 14:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, 18:3 and 20:4. No identification of the other trace peaks was attempted. Although identified 10:0 represented less than 0.1% of the total in 83% of the samples and never more than 0.3%. Accordingly values for this acid were not analysed further.

The unknown peak, originally thought to be 20:0 represented from a trace to as much as 1.7% of the total accordingly has been taken into consideration in the calculations.

Representative mean results for individuals of each sex at different ages in anterior abdominal wall fat are given in Table I. General trends are discernible in these selected figures but analysis of this amount of data proved to be highly complex.

A two-way computer analysis of covariance with one observation (i.e. the mean) per cell



structure by comparison with standards of known composition. Although other esters such as those of cholesterol or phospholipids might contribute to the fatty acids in the mixture it was felt that such an effect would be negligible since over 99% of adipose tissue lipid has been shown to be triglyceride (20).

Utilising NIH standards A, B, C and D replicate samples agreed with the stated composition to within 3% for major peaks (greater than 10% of the total) and within 3.5% for minor peaks (less than 10% of the total) (16).

The analytic system could not readily distinguish

between the peaks of the 18:3 and 20:0 esters and accordingly the level for the former included trace of the latter. A minor peak originally identified as 20:0 was found to be indistinguishable from it and has not been positively identified.

Several trace peaks (defined as less than 0.1% of the total) were consistently seen. They were not included in the calculations as collectively they would not influence the results significantly. Initially kept

The number before the colon refers to the chain length that after to the degree of unsaturation.

Table 3 Fatty acid composition of milk triglycerides

Composition expressed as percentage

	4 0	6 0	8 0	10 0	12 0	14 0	16 0	16 1	18 0	18 1	18 2	20 0
Fatty acid	10.2	2.5	1.3	1.5	3.3	8.6	21.1	2.8	9.9	31.4	4.9	0.7
Bovine milk	—	—	—	2.1	7.7	9.0	23.6	3.4	6.5	33.2	7.1	0.9
Human milk	—	—	—	—	—	—	—	—	—	—	—	—

Bovine data from Hilditch &amp; Paul 1940. Human data from Hilditch &amp; Meern 1944. See also Hilditch &amp; Williams (12).

Only a few papers have examined trends throughout childhood (1, 8, 23, 34) and these in general have shown that the proportion of 16:0 and sometimes of 16:1 and 18:0 is much higher in depot fat from newborns than in older children while the proportion of 18:2 is lower. Takazawa (34) reports that the ratio of unsaturated to saturated fatty acid content increases with increasing age. When considering these findings it must be remembered that since the figures are always expressed as percentages a change in the level of one component particularly a major one inevitably changes the values for all the rest: this adds to the complexities of analysing this type of data.

Fujimoto (8) showed a continuing but gradual change from infancy until about 7 or 8 years of age by which time values similar to the adult were reached.

The present results confirm in general the reported values but extend them. Levels of 12:1 and 18:2 are strikingly higher in infancy; levels of 14:0 and 16:0 are higher and levels of 18:1 are much lower than in older children while levels of 18:3 are somewhat lower. Most of the trend toward adult levels appears to have occurred by about 5 years of age. Levels of 20:4 seem highly variable.

An age trend in adults was reported by Insull & Bartusch (18). In a large series ranging from adolescence to 79 years the proportion of 18:1 was said to increase with age while that of 12:0, 14:0 and 18:1 tended to decrease. Such observations may influence the interpretation of conclusions reached in adult studies on small populations.

**Sex.** The majority of reports in the literature did not consider a possible effect of sex on com-

position. McLaren *et al.* (26) did not observe any sex difference in a small and heterogeneous population. Pietropolo *et al.* (31) in a study of adults concluded that "depot fat in man presents an almost uniform make up. Although they make no specific comment their data shows no sex effect."

Krot & Bronie Stewart (24) studying adults of varying racial origin reported that males had significantly higher content of 16:0 and 18:0 in their depot fat while females had more 16:1 and 18:1. However these sex differences varied in the various racial groups studied. Since at least 16:0 and 18:1 are major components of depot fat these observations would suggest that female fat is more unsaturated than is "softer" than male since unsaturation for a given chain length reduces melting point.

Insull & Bartusch (18) found in their adult autopsy specimens that males had slightly higher 18:0 content than females ( $p < 0.05$ ) while females had more 18:1 (n.s.). In view of the common failure to take factors such as age and in some cases sampling site into consideration drawing definite conclusions on the basis of published figures is hazardous and the possibility of sex differences in adults or in children remains.

**Site.** Many authors had sampled from only one site or did not report the sites from which the samples were taken. We have not investigated in this present study possible differences in composition between superficial and deep depot fat.

Iwachi *et al.* (17) reported that distal limb sites showed significant compositional differences from abdominal wall sites, the latter having more 14:0, 16:0 and 18:0 and less 14:1 and

Table 1 Analyses of superficial fat from typical subjects at different ages (mean values)

Acid	12 0	14 0	16 0	16 1	17 0	18 0	18 1	18 2	18 3	20 4
<i>Males</i>										
3 months	8.1	7.5	21.6	4.3	0.0	2.7	23.0	28.7	1.4	0.6
1½ years	1.9	5.5	21.0	5.4	0.6	7.7	40.4	10.2	3.3	1.3
3 years	1.3	4.6	22.3	4.0	0.7	10.5	40.7	7.8	2.2	1.4
5 years	0.8	3.0	16.4	4.3	0.6	8.1	51.5	11.0	3.6	2.6
15 years	0.6	3.2	16.6	5.9	0.0	7.3	45.4	9.7	5.9	3.9
<i>Females</i>										
2 months	7.8	6.2	17.4	3.1	0.3	3.0	25.3	31.3	1.1	0.7
17 months	1.4	3.3	17.6	4.6	0.6	7.9	43.6	13.1	2.8	0.7
3 years	0.8	4.1	18.9	5.6	0.7	8.0	42.4	8.4	4.2	3.0
5 years	0.7	3.5	17.8	9.2	0.7	3.7	48.2	9.8	3.4	0.0
14 years	0.5	2.7	16.7	10.8	0.0	2.5	51.1	9.1	3.2	0.7

was undertaken on the IBM 360 computer taking into account age sex and subcutaneous site (i.e. abdominal wall or limb). Unfortunately as the data were of course collected in a random fashion cell sizes were quite unequal, which tended to reduce the potency of the mathematical technique. Since making the cell size more equal, either by reducing the cells to the lowest common value or selectively accumulating further data to enlarge the smaller cells would also disturb the validity of the method no changes have been made. It is possible that minor degrees of correlation may have been missed as a consequence.

*Effect of age* The levels of 12.0, 14.0, 16.0 and 18.2 are much higher in infancy than in adult life and diminish in most instances rapidly at first and then more slowly in mid childhood. Conversely the level of 18.1 is

much lower in infancy and rises thereafter. The regression coefficients for these acids by age (sex and site being kept constant) are shown in Table 2. Although the curve of mean values would frequently be better represented by a curve the complexity of analysis required was not felt to be justified and so straight lines have been fitted.

*Effect of sex* No effect of sex on composition could be detected when the other variables were controlled.

*Effect of subcutaneous site* No difference in composition between samples from anterior abdominal wall and from limb sites was demonstrated. This is at variance with some evidence in the literature and may reflect the fact that most limb samples were from relatively proximal limb sites.

## DISCUSSION

*Age* The topic of human depot fat composition has received considerable attention in recent years and although the majority of reports deal with samples from adult subjects (10, 17, 18, 22, 24, 31) several have dealt with samples from children particularly in the newborn period. In two reports the age of the subjects was not specified (21, 27) but was assumed to be adult. Dramatic differences have been shown between the composition of depot fat in the newborn as compared to either normal adult controls (1, 3, 26) or the infant.

Table 2 Regression data for fatty acid composition

Fatty acid	Slope $s$ ( per year)	Intercept $t$ ( )
12 0	-0.10039	1.885
14 0	-0.14164	4.966
16 0	-0.21138	20.741
16 1	0.09031	5.460
17 0	-0.01301	0.704
18 0	-0.09281	7.171
18 1	0.50834	39.740
18 2	-0.11522	12.052
18 3	0.07038	2.875
20 4	0.02267	1.274

$$y = \text{fatty acid percentage} \quad x = \text{age in years} \quad y = sx + t$$

Table 3 Fatty acid composition of milk triglycerides

Composition expressed as percentage

	4.0	6.0	8.0	10.0	12.0	14.0	16.0	16.1	18.0	18.1	18.2	20.0
Bovine milk	10.2	2.5	1.3	1.5	3.3	8.6	21.1	2.8	9.9	31.4	4.9	0.7
Human milk	—	—	—	2.1	7.7	9.0	23.6	5.4	6.5	33.2	7.1	0.9

Bovine data from Hiddeh &amp; Paul 1940. Human data from Hiddeh &amp; Meern 1944. See also Hiddeh &amp; Williams (12).

Only a few papers have examined trends throughout childhood (1, 8, 23, 34) and these in general have shown that the proportion of 16:0 and sometimes of 16:1 and 18:0 is much higher in depot fat from newborns than in older children while the proportion of 18:2 is lower. Takazawa (34) reports that the ratio of unsaturated to saturated fatty acid content increases with increasing age. When considering these findings it must be remembered that since the figures are always expressed as percentages a change in the level of one component, particularly a major one, inevitably changes the values for all the rest, thus adding to the complexities of analysing this type of data.

Fujimoto (8) showed a continuing but gradual change from infancy until about 7 or 8 years of age by which time values similar to the adult were reached.

The present results confirm in general the reported values but extend them. Levels of 17:0 and 18:2 are strikingly higher in infancy, levels of 14:1 and 16:0 are higher and levels of 18:1 are much lower than in older children while levels of 18:3 are somewhat lower. Most of the trend towards adult levels appears to have occurred by about 5 years of age. Levels of 20:4 seem highly variable.

An age trend in adults was reported by Insull & Bartsch (18). In a large series ranging from adolescence to 79 years the proportion of 18:1 was said to increase with age while that of 12:0, 14:0 and 18:0 tended to decrease. Such observations may influence the interpretation of conclusions reached in adult studies on small populations.

Sex. The majority of reports in the literature did not consider a possible effect of sex on com-

position. McLaren *et al.* (26) did not observe any sex difference in a small and heterogeneous population. Pietropaolo *et al.* (31) in a study of adults concluded that depot fat in man presents an almost uniform make up. Although they make no specific comment their data shows no sex effect.

Krut & Bronte Stewart (24) studying adults of varying racial origin reported that males had significantly higher content of 16:0 and 18:0 in their depot fat while females had more 16:1 and 18:1. However these sex differences varied in the various racial groups studied. Since at least 16:0 and 18:1 are major components of depot fat these observations would suggest that female fat is more unsaturated than is male fat since unsaturation for a given chain length reduces melting point.

Insull & Bartsch (18) found in their adult autopsy specimens that males had slightly higher 18:0 content than females ( $p < 0.05$ ) while females had more 18:1 (n.s.). In view of the common failure to take factors such as age and in some cases sampling site into consideration drawing definite conclusions on the basis of published figures is hazardous and the possibility of sex differences in adults or in children remains.

Site. Many authors had sampled from only one site or did not report the sites from which the samples were taken. We have not investigated in this present study possible differences in composition between superficial and deep depot fat.

Insull *et al.* (17) reported that distal limb sites showed significant compositional differences from abdominal wall sites, the latter having more 14:0, 16:0 and 18:1 and less 14:1 and

16:1 than the former. They speculate that the differences may be related to physical properties required by the site: more peripheral and thus environmentally cooler sites bearing fat of lower melting point (more unsaturated) than central.

Unfortunately the data are drawn from a very small sample (1 female and 5 male adults of widely disparate ages). They conclude that since all the fatty acids concerned can be synthesised *de novo* by the adipose tissue, the differences may be related to different synthetic preferences between the tissues.

Other authors (10, 26, 27) report only that leg depot fat has less 18:0 and more 16:1 than abdominal wall fat, the composition being otherwise similar.

**Other Factors.** That diet can influence depot fat composition is well established (13, 14). At least in the adult, the effect of a major change in dietary fat composition is reflected only very slowly in the depot fat. Hirsch (13) estimates the half time of exchange between dietary (and therefore plasma (7)) and depot triglyceride fatty acids at 350–750 days. However, in periods of rapid weight gain such as early infancy or during re-alimentation after severe malnutrition, much more rapid changes can occur. Feeding corn oil to a nursing mother produced immediate changes in maternal milk composition and the infant's depot fat reflected the change within 10 days (14). Sweeney (33) has also observed the same trend in small infants during rapid weight gain.

McLaren *et al.* (16) however, on the basis of their studies in different ethnic groups in Africa, doubt that diet is a primary factor in depot fat composition. The subjects studied by Pietro Paolo *et al.* (31) in Italy had higher levels of 18:1 than usually reported, and this was felt to be a dietary effect.

After the period of infancy (say the first year of life) most children in North America consume foods more or less identical with those of the adults of the family.

Accordingly, if we are to seek dietary factors to explain the gradation of composition

through childhood, it is more likely a quantitative rather than a qualitative effect. The most obvious possibility is, of course, the consumption of bovine milk. Children consume much more milk than adults, many of whom consume very small amounts only.

The composition of typical bovine and human milk fats are shown in Table 3. It will be observed that relative to adult depot fat composition, bovine milk contains more 12:0, 14:0 and 16:0 but less 18:1 and 18:2. Compared with infant depot fat, bovine milk contains rather less 12:0 (although the difference could be accounted for by lengthening the chain of some of the shorter fatty acids prominent in milk) about the same amount of 14:0 and 16:0, much more 18:0 and about the same amount of 18:1. Thus far the relationship appears promising, but milk contains much less 18:2 than depot fat of infants, which is particularly rich in this essential fatty acid. Of course, if chain length alteration is invoked to explain such differences, the significance of the similarity between diet and depot fat is lost.

Little is known about hormonal influences. Sex differences in fatty acid metabolism have been reported (4, 9). There is no evidence of selective mobilization of any specific fatty acids in response to lipolytic agents (30).

Environmental temperature might be a factor, as suggested by Henriques & Hansen in the hog (11) and Imachi *et al.* (17) in man. Several studies from Africa have claimed that environmental temperature changes are responsible for differences in depot fat composition (25, 26).

In view of the dearth of unequivocal data regarding the effect of factors other than diet on depot fatty acid composition, speculation regarding the significance of any of such composition remains speculative. Since there seems little doubt that a major proportion of the fatty acids in the mammalian system are synthesised from dietary carbohydrate sources (26), just why dietary fatty acid patterns should apparently influence composition so much is difficult to explain. Foods rich in carbohydrate are said to

favour the deposition of saturated fatty acids especially stearic (18:0) (20:1, 35). Indeed in the human adult although adipose tissue is a major site of lipogenesis from glucose at times of glucose excess at other times the liver appears predominant (2). Further in adipose tissue glyceride glycerol is probably the major product rather than fatty acid (2, 15). Whether this holds true in the metabolically more active child is not established.

Studies in the last decade have suggested that depot fat is not a torpid storehouse for excess calories but an active metabolic system providing rapid fluxes of energy either as fatty acids or glucose inwards in times of alimentary and outwards as fatty acids and glycerol in times of fasting or great metabolic need. If this is so it is surprising that dietary changes should take such a very long time to affect composition. This might suggest compartmentalisation of the depots with a large inert mass and small active one. There is no evidence of such differentiation in man.

Recent studies have shown that there are fatty acid families possibly important in the mammalian economy which were previously unrecognized (32). It is clear that future studies must concentrate on the relationship of the cellular environment to the composition of the fat being stored rather than just the static composition thereof.

## SUMMARY

In childhood age has a major influence on the fatty acid composition of superficial adipose depot triglycerides. Although the effect is most marked in the newborn period it continued at least to mid-childhood. After correcting for this variable no difference between male and female was apparent and no significant difference was observed between anterior abdominal wall and limb fat. It seems unlikely that diet alone can explain these differences. Our understanding of other possible factors in the determination of fatty acid distribution in stored fat is meagre.

## ACKNOWLEDGEMENTS

The meticulous assistance of Miss R. Black and Mrs M. Don Paul in the analyses and the aid of Dr H. T. Davenport in the collection of samples was invaluable. The mathematical treatment of the results would not have been possible without the generous aid of Dr B. M. Alfred. Mr R. Meldrum kindly provided the computer plots.

The study has been supported by funds from the Medical Research Council of Canada (MA 2467).

## REFERENCES

1. Baker G. L. Human adipose tissue composition and age. *Am J Clin Nutr* 22: 829, 1969.
2. Björntorp P., Schersten T., Gottfrid A. Effects of glucose infusions on adipose tissue lipogenesis in man. *Acta Med Scand* 183: 656, 1968.
3. Bondrup K., Lyster N., Quasid F., Rasmussen H. Gas chromatography analyses of human subcutaneous fat. *Scand J Clin Lab Invest* 18: 583, 1966.
4. Christensen K., Gen M. V., Holman R. T. Sex differences in the metabolism of fatty acids in vitro. *Biochim Biophys Acta* 187: 19, 1969.
5. Dawkins M. J. R., Stevens J. T. Fatty acid compositions of triglycerides from adipose tissue. *Nature* 209: 1145, 1966.
6. Endres J. G. An apparatus for the rapid preparation of fatty acid esters from lipids for gas chromatography analysis. *J Amer Oil Chem Soc* 43: 600, 1966.
7. Flinckham A. I., Rayton T., Beresbaum A. L. Variations in the composition of free fatty acids with dietary change under metabolic conditions. *Am J Clin Nutr* 15: 299, 1964.
8. Fujimoto E. Studies on the fatty acid composition of body fat with particular reference to body weight and age. *Acta Paediat Jap* 8: 36, 1966.
9. Gilmour R. F., McEwen K. W. Insulin and tropic regulation of lipid synthesis in adipose tissue. *Biochim Biophys Acta* 116: 220, 1966.
10. Hellerman A. O. A. The fatty acid composition of human adipose tissue. *Clin Sci* 75: 433, 1963.
11. Henriques V. A., Hansen C. Comparative studies on the chemical composition of animal fat. *Scand Arch Physiol* 11: 151, 1901.
12. Hilditch T. P., Williams P. N. *The chemical composition of natural fats*. Chapman and Hall, London, 4th edn, 1964, p. 118.
13. Hirsch J. Fatty acid patterns in human adipose tissue. In *Handbook of Physiology*, Section 5, Adipose Tissue. Amer Physiol Soc, 1956, p. 181.
14. Hirsch J., Farquhar J. W., Ahrens E. H., Peterson M. L., Stoffel W. Studies of adipose tissue in man. *Am J Clin Nutr* 8: 499, 1960.
15. Hirsch J., Goldrick J. B. Serial studies on the metabolism of human adipose tissue. I. *J Clin Invest* 43: 1776, 1964.



- 6 Hornung H C Ahrens E H Lipsky S R Mattson F H Mend J F Turner D A & Goldwater W H Quantitative analysis of fatty acids by gas liquid chromatography *J Lipid Res* 5 20 1964
- 7 Imachi K Fukuda J Oyama K & Mukawa, A Effect of sample site on fatty acid composition of adipose tissue *J Biochem (Tokyo)* 58 463 1965
- 8 Insall W & Bartoch G E Fatty acid composition of human adipose tissue related to age and race *Am J Clin Nutr* 20 13 1967
- 9 James A T & Martin A J P Gas liquid chromatography the separation and identification of the methyl esters of unsaturated and saturated fatty acids from formic acid to n octadecanoic acid *Biochem J* 63 44 1956
- 10 Jeanrenaud B Lipid components of adipose tissue In *Handbook of Physiology* Section 5 Adipose Tissue Am Physiol Soc 1956 p 169
- 11 Kingsbury K J Morgan D M & Heyes T D The analysis of the fatty acids of normal human depot fat by gas liquid chromatography *Biochem J* 90 140 1964
- 12 Kingsbury K J Paul S Crossley A & Morgan D M The fatty acid composition of human depot fat *Biochem J* 78 341 1961
- 13 Kondo T Studies on the fatty acid composition of the bone marrow fat in childhood *Acta Paediat Jap* 9 86 1967
- 14 Krut L H & Bronte Stewart B The fatty acids of human depot fat *J Lipid Res* 5 343 1964
- 15 MacDonald I Ambient temperature and depot fat iodine number of children *Nature* 192 353 1961
- 16 McLaren D S Ajam Z A & Awdeh Z Composition of human adipose tissue with special reference to site and age differences *Am J Clin Nutr* 17 171 1965
- 17 McLaren D S & Read W W C Fatty acid composition of adipose tissue *Clin Sci* 23 247 1962
- 18 Martinsson A On the composition of human adipose tissue *Acta Med Scand* 182 795 1967
- 19 Metcalfe L B Schmidz A A & Pella J R Rapid preparations of fatty acid esters from lipids for gas chromatography analysis *Analyt Chem* 38 515 1966
- 20 Norcia L N & Evans J D Relation of plasma free fatty acid composition to the origin of free fatty acids from depot fat triglycerides *Metabolism* 13 12 1964
- 21 Pietropaolo C Pasano, L & Cah G Fatty acid composition of human adipose tissue in different sites *Clin Chim Acta* 10 485 1964
- 22 Schlenk H & Sand D M A new group of essential fatty acids and their comparison with other polyenoic fatty acids *Biochim Biophys Acta* 144 305 1967
- 23 Sweeney M J Etteldorf J N Throop L J Timma D L & Wrenn E L Diet and fatty acid distribution in subcutaneous fat and in the triglycerides cholesterol fraction of serum of young infants *J Clin Invest* 42 1 1963
- 24 Takazawa Y Studies on the fatty acid composition of subcutaneous fat in childhood *Acta Paediat Jap* 9 87 1967
- 25 Vague P The degree of masculine differentiation of obesees a factor determining predisposition to diabetes atherosclerosis gout and uric calculous disease *Am J Clin Nutr* 4 1 1956
- 26 Wilson R H Doty V E Rencz A H & Schram A C Gas chromatographic analysis of high molecular weight fatty acid methyl esters with the technique of relative molar response *J Lab Clin Med* 67 77 1966

Submitted Nov 11 1969

Accepted Jan 20 1970

Children's Hospital  
250 West 59th Avenue  
Vancouver 155  
British Columbia  
Canada

Key words Depot fat triglyceride fatty acids

## MAXIMUM TUBULAR REABSORPTION OF WATER

### *A Preliminary Study of Renal Concentrating Capacity in Children with Chronic Pyelonephritis*

H. C. SOMMERSCILD and G. GROTTÉ with technical assistance  
by KATARINA HALLIN

*From the Department of Pediatric Surgery, University Hospital, Uppsala, Sweden*

The concentrating capacity is assumed to be the first of the kidney functions affected in recurrent urinary infections. This capacity is usually expressed as maximum urinary specific gravity during 14-16 hours of hydration. Winberg (15) has shown that normal osmolality should exceed 800 mOsm/l and that children reach this capacity during the third year of life. However, these concentration tests based upon urinary specific gravity measurements have a significant rate of error.

Another method of determining renal concentration capacity is by induced osmotic diuresis or hydropenic acid diuresis. Osmotic diuresis is effected by intravenous infusion of a substance such as mannitol which is filtered in the glomerulus but is not reabsorbed in the nephrons. In this way the ability of the kidney to build up interstitial medullary hyperosmolality may be tested. This may be expressed as osmotic clearance (Co<sub>sm</sub>) using the total amount of cleared osmotically active substances as the classical definition of renal clearance. The more effective this ability is, the greater the amount of water reabsorbed from the collecting tubules will be. The amount of water reabsorbed in excess of the amount corresponding to the reabsorbed osmotically active substances is termed "reabsorbed free water" ( $\Delta V_{H_2O}$ ). In isosthenuria the magnitude of Co<sub>sm</sub> and diuresis is the same and  $\Delta V_{H_2O} = 0$ . Each increase in diuresis is accompanied by an equal increase in Co<sub>sm</sub> thereby creating an isosthenic curve or base line (Fig. 1) (see infra). These parameters may be expressed as follows:

The purpose of this investigation was to determine the possibility of detecting impaired renal concentration capacity at an earlier stage by use of mannitol induced osmotic diuresis than is possible by the conventional specific gravity measurements.

## MATERIAL AND METHODS

Eleven patients from 2-13 years of age were investigated. Ten of these patients had recurrent urinary infections with acute relapses during the 1-5 year observation period. Four of these ten showed normal renal function as far as could be assessed; three had depressed concentrating capacity of varying severity and three had stoneuria and marked papillary changes in IVP (Table 1). No urinary system obstruction and no residual urine were observed in these patients. They were all referred to the pediatric surgical clinic for evaluation of surgical intervention. One patient was treated for hypospadias.

**Method.** At 6:00 p.m. on the day before the investigation the patient was given verapresin (Pitresin tablets in oil, Parke Davis) 0.5-1 U/kg body weight i.m. Fluids were then withheld during the following 14 hours. In the morning an indwelling bladder catheter was connected to a sample bottle. At 8:00 a.m. the urine was collected for 30 min. During the next 2 hours 10% mannitol was intravenously infused at a constant rate of 3 mOsm/kg/hour. Urine specimens were collected at 15 min intervals and a blood sample taken at the same time.

**Analysis.** The total osmolality in the urine and serum was estimated by the freezing point technique using a Rastmeyer osmometer. Sodium and potassium in the blood and urine were analyzed by flame photometry while chloride and creatinine were determined according to the Technicon autoanalyzer methodology.

- 16 Hornung E C Ahrens E H Lipsky S R Mattson F H Mead J F Turner D A & Goldwater W H Quantitative analysis of fatty acids by gas liquid chromatography *J Lipid Res* 5 20 1964
- 17 Imachi K Fukuda J Oyama K & Mukawa A Effect of sample site on fatty acid composition of adipose tissue *J Biochem (Tokyo)* 58 463 1965
- 18 Insull W & Bartsch G E Fatty acid composition of human adipose tissue related to age sex and race *Am J Clin Nutr* 20 13 1967
- 19 James A T & Martin A J P Gas liquid chromatography the separation and identification of the methyl esters of unsaturated and saturated fatty acids from formic acid to n-octadecanoic acid *Biochem J* 63 44 1956
- 20 Jeanrenaud B Lipid components of adipose tissue In *Handbook of Physiology Section 5 Adipose Tissue* Am Physiol Soc 1956 p 169
- 21 Kingsbury K J Morgan D M & Heyes T D The analysis of the fatty acids of normal human depot fat by gas liquid chromatography *Biochem J* 90 140 1964
- 22 Kingsbury K J Paul S Crowley A & Morgan D M The fatty acid composition of human depot fat *Biochem J* 79 541 1961
- 23 Kondo T Studies on the fatty acid composition of the bone marrow fat in childhood *Acta Paediat Jap* 9 86 1967
- 24 Krut L H & Bronte Stewart B The fatty acids of human depot fat *J Lipid Res* 5 343 1964
- 25 MacDonald I Ambient temperature and depot fat iodine number of children *Nature* 192 353 1961
- 26 McLaren D S Ajum Z A & Andeh Z Composition of human adipose tissue with special reference to site and age differences *Am J Clin Nutr* 17 171 1965
- 27 McLaren D S & Read W W C Fatty acid composition of adipose tissue *Clin Sci* 23 247 1962
- 28 Martinsson A On the composition of human adipose tissue *Acta Med Scand* 182 795 1967
- 29 Metcalfe L D Schmdz A A & Felka J L Rapid preparations of fatty acid esters from lipids for gas chromatography analysis *Anal Chem* 38 515 1966
- 30 Norcia L N & Evans J D Relation of plasma free fatty acid composition to the origin of the fatty acids from depot fat triglycerides *Metabolism* 13 12 1964
- 31 Pietropaolo C Pisano L & Cah U Fatty acid composition of human adipose tissue in different sites *Clin Chim Acta* 10 485 1964
- 32 Schlenk H & Sand D M A new group of essential fatty acids and their comparison with other polyenoic fatty acids *Biochem Biophys Acta* 144 305 1967
- 33 Sweeney M J Etteldorf J N Throop L J Tumma D L & Wrenn E L Diet and fatty acid distribution in subcutaneous fat and in the triglycerides cholesterol fraction of serum of young infants *J Clin Invest* 42 1 1963
- 34 Takizawa Y Studies on the fatty acid composition of subcutaneous fat in childhood *Acta Paediat Jap* 9 87 1967
- 35 Vague P The degree of masculine differentiation of obesities a factor determining predisposition to diabetes atherosclerosis gout and uric calculous disease *Am J Clin Nutr* 4 1 1956
- 36 Wibon R H Doty V E Rencz K H & Schram A C Gas chromatographic analysis of high molecular weight fatty acid methyl esters with the technique of relative molar response *J Lab Clin Med* 67 87 1966

Submitted Nov 11 1969

Accepted Jan 20 1970

Children's Hospital  
230 West 59th Avenue  
Vancouver 155  
British Columbia  
Canada

Key words Depot fat triglyceride fatty acids

Table 2 Highest and lowest value of different parameters during the investigation

No	Age year	Na/s mEq/l Max-Min	Osm/s mOsm/l Min-Max	Osm/urine mosm/l Max-Min	Diuresis ml/min/73 m Min-Max	$C_{max}$ ml/m/73 m
1	3	138-123	270-350	720-420	0.6-6.6	15.6
2	5	136-127	280-340	810-540	1.0-5.0	9.0
3	5	137-123	270-295	980-515	0.8-7.6	13.5
4	7	145-130	290-308	970-550	1.0-5.6	10.6
5	5	147-124	307-331	1010-538	0.5-5.0	8.5
6	2	140-129	270-300	660-422	0.7-5.7	8.5
7	3	144-124	264-333	750-270	0.7-4.4	5.2
8	4	144-120	290-350	540-370	0.7-5.1	7.1
9	2	140-127	280-333	400-315	0.9-6.0	6.0
10	5	148-137	290-350	320-120	0.7-7.4	7.0
11	13	140-125	280-345	320-370	1.2-6.3	6.3

Minimum diuresis refers to the 30 min. period before starting mannitol infusion.  
Maximum urinary concentration also refers to this period.

but should always remain above a minimum level. In healthy individuals a stable level of  $T_{H_2O}$  is found when the diuresis is 4-5 ml/min/73 m<sup>2</sup> BSA or more (vide infra).  $T_{H_2O}$  tends to decrease with an extremely large diuresis of 15-20 ml/min or more (5).

Fig. 1 shows the normal variation in patients examined by Whitten & Younes (14) which conforms with the results in other investigations (1 & 5, 11, 16). Based upon the results reported in the literature, values of  $T_{H_2O}$  max below 3.2 ml/min/73 m<sup>2</sup> BSA must be considered abnormal. In most cases  $C_{osm}$  and diuresis are equally large and  $T_{H_2O} = 0$  (baseline).  $T_{H_2O}$  appears as the horizontal distance between the base line and the patient's curve and the greater the distance the larger the  $T_{H_2O}$ . When  $T_{H_2O} > 3.2$  ml/min the patient's curve will fall in the shaded normal area. If the curve falls between this area and the base line  $T_{H_2O}$  is pathologically low.

Investigations of this kind have been carried out on adults without kidney disease, on adults with acute and chronic renal failure and on patients in the early post-operative period (2, 4, 5, 6, 10, 11, 12, 13, 16). Rapoport *et al.* (12) have examined children 8-15 years of age without renal disease. Whitten & Younes have done a comparative study of 12 healthy children and 15 with sickle cell anemia, all within the 1-15 year age group. Hatch *et al.* (7) and Penhale *et al.* (9) have also described investigations in patients with sickle cell anemia.

We have been unable to find any other work dealing with the renal concentrating ability in adults or in children with recurrent urinary infections as evaluated by  $C_{osm}$  and  $T_{H_2O}$ . The results of our investigation, however, correlate well with similar studies carried out on patients with other diseases. Our material is too small for definite conclusions but the results indicate that the method is helpful in detecting impaired renal concentrating capacity at an early stage. A moderately depressed concentrating capacity in patients with normal maximum urinary concentration may possibly be revealed by this method. However, the most important application of this method may be in the cases where the conventional concentrating test is inconclusive. Here estimation of  $C_{osm}$  and  $T_{H_2O}$  may be of value.

## SUMMARY

In an original study the renal concentrating capacity in ten children with recurrent urinary infections and in one patient with hypospadias has been estimated by maximum urinary specific gravity followed immediately by calculation of  $C_{osm}$  and  $T_{H_2O}$  during mannitol infusion. The normal values found here correlate well with those reported in the literature. The results indicate that estimation of  $C_{osm}$  and  $T_{H_2O}$  may reveal impaired renal concentrating capacity at an earlier stage than does the conventional test of maximum urinary specific gravity. The method

Cosm  $\frac{\text{Umm osm}}{\text{Serum osm}}$  univol  
 $T_{H_2O}$  Cosm univol

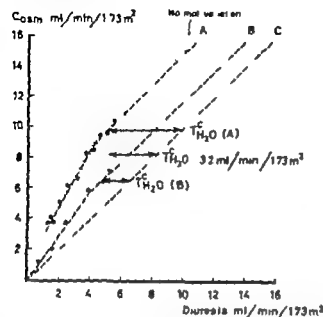


Fig. 1 The area of normal variation of  $T_{H_2O}$  according to Whitten & Younes. The A line is based upon the values from patient no. 4 as an example of the normals. The B line refers to patient no. 6 with pathological value for  $T_{H_2O}$  but no isostenuria. The C line represents the isostenuric patients (zero line).

**Calculations.** The mannitol dosage was adjusted according to the body weight. All other calculations were adjusted to body surface area (BSA) of  $1.73 \text{ m}^2$ .

## RESULTS

Four patients showed normal values for all parameters. Four patients with definite reduction in urinary concentrating capacity revealed pathological  $T_{H_2O}$  values. Two patients showed conclusive levels of maximum urinary concentration. In these two patients the  $T_{H_2O}$  slope was obviously subnormal, however, suggesting that the concentrating capacity was in fact affected. The hypostatic patient had normal  $T_{H_2O}$  values. These results are shown in Table 1. Table 2 shows the actual variations in serum sodium, serum osmolality, urinary osmolality, diuresis and Cosm throughout the investigation. The  $T_{H_2O}$  slopes plotted in Fig. 1 represent the three groups of patients: normal, isostenuric and intermediate as well as the area of normal variation.

## DISCUSSION

During mannitol-induced osmotic diuresis, the tubular reabsorption of water ( $T_{H_2O}$ ) increases with the increasing diuresis. When the diuresis exceeds a critical value, the slope for  $T_{H_2O}$  tends to parallel the isostenuric baseline. This indicates that  $T_{H_2O}$  has become stable and the value obtained is called  $T_{H_2O} \text{ max}$ . This value varies from patient to patient and may also vary in certain patients from one examination to the

Table 1 Mean  $T_{H_2O}$  for the individual patients correlated to maximum urinary concentration and other laboratory investigations as well as to the clinical course

No	Age year	History	X-ray	Isotope renogram	Creat/s mg/100 ml	Max urine concent mOsm/l	Level of $T_{H_2O} \text{ max}$ ml/min/1.73 m <sup>2</sup>
1	3	N	N	O	0	720	3.7
2	5	(+)	N	N	0.7	810	3.6
3	5	-	(P)	N	0.8	980	3.4
4	7	+	P	N	0.8	970	4.3
5	5	-	PP	N	1.0	1010	3.4
6	2	+	P	N	0.8	660	2.6
7	3	+	PP	P	0.8	750	0.6
8	4	+	(P)	E	0.8	540	1.8
9	2	++	PP	P	1.5	400	1.6
10	5	++	PP	F	0.8	320	0.0
11	13	++	PP	N	2.0	320	0.0

N = normal (P) P PP = pathological findings of increasing severity

O = not performed

- = no clinical symptoms when on therapy with antibacterial drug

(+) + ++ = relapses of acute infections of increasing severity during therapy

## SALICYLATE INDUCED FOETAL DAMAGE DURING LATE PREGNANCY IN MICE

*The modifying effect of repeated administration and dosage*

MARGARETA ERIKSSON

*From the Laboratory of Teratology and the Department of Paediatrics  
Karolinska Institute, Stockholm, Sweden*

For many years teratological experiments have been based on the well known fact that gross malformations can be induced by exogenous factors during organogenesis (2). Thus the early tests for safety of new drugs were designed in agreement with this fact (27). Attention is now being paid to the vulnerability of the foetus in the perinatal period and special recommendations regarding this point have recently been included in the new guidelines (13, 24). There are however only a few experimental reports available. Earlier studies from our laboratory have shown salicylate to produce a variety of foetal injuries when administered late in pregnancy (8, 9, 10, 18).

There has been increasing interest in perinatal pharmacology during the last decade (7). Experience from investigations of drug metabolism late in pregnancy will be of great value for the design of perinatal drug tests. The present study was undertaken to investigate the role of dosage and the frequency of administration on the prenatal effect produced by salicylate. During this study special interest was devoted to the possibility of simultaneously influencing drug metabolism and the foetal damaging effect.

### MATERIAL AND METHODS

All mice raised in our laboratory have been inbred and kept as described earlier (16). Drugs were

administered at 10 a.m. in a volume of 0.1 ml distilled water per 20 g body weight. Sodium salicylate as i.m. and pentobarbital as i.p. injections.

The LD 50 for sodium salicylate was determined in 25 adult virgin females. They were given different doses between 12 and 18 mg per 20 g body weight. Statistical calculation was made according to the method described by Litchfield & Wilcoxon (19).

The effect of different salicylate-doses on the foetus late in pregnancy was examined in 40 pregnant mice (Table 1). Sodium salicylate was given as single injections in a dose of 3 mg/10 mg or 15 mg/20 g on day 17 of gestation. The day on which vaginal plug was observed is denoted as day zero of pregnancy. Untreated animals of the same gestational age served as controls. The females were observed daily for premature delivery or maternal death. On day 18 at 10 a.m. undelivered females were sacrificed and the foetuses removed for examination. The number of living and dead foetuses and the developmental stages of the resorbed ones were recorded. Foetal resorptions which had evidently occurred before the mother had received the injection are not included in the tables. Living foetuses were examined macroscopically for gross malformations and for superficial and intra-abdominal haemorrhage as described earlier (9, 17).

The effect of single and repeated administration of salicylate was compared in 80 animals (Table 2). Three groups were given sodium salicylate as a single dose of 10 mg/20 g on gestation day 15, 16 or 17. A fourth group received 10 mg/20 g on day 15, 16 and 17. Another group was injected with 3 mg/20 g on day 15 and 16 of gestation and 10 mg/20 g on day 17. The observations of foetal damage were made as described in the dose experiment above, but foetal resorption which had occurred before the earliest injection, i.e. day 15, is not included.

The effect of salicylate after pretreatment with pentobarbital was studied in 30 animals (Table 3). One group of 20 was given pentobarbital (Nemba-

therefore seems valuable as a supplement to the conventional test in selected cases where early detection of beginning deterioration of the renal concentrating capacity is important

# ACKNOWLEDGEMENT

This investigation was supported by grants from Olle and Elof Ericssons Stiftelse Årvidaberg Sweden. The authors want to thank Prof C H deVerdier MD and Olof Ericsson laboratory engineer at the Laboratory for Clinical Biochemistry Akademiska Sjukhuset Upsala who have carried out all the laboratory analysis necessary for this investigation

# REFERENCES

- 1 Alken C E, Dux V W, Wegrauch H M & Wildbolz E. *Handbuch der Urologie*. Springer Verlag Berlin 1965 vol II p 236
- 2 Barry K G, Cohen A & LeBlanc P. Mannitolization I. The prevention and therapy of oliguria associated with cross clamping of abdominal aorta. *Surgery* 50 353 1961
- 3 Edelman C M Jr, Barnett H L, Stark H, Boichus K & Soriano J B. A standardized test of renal concentrating capacity in children. *Amer J Dis Child* 114 639 1967
- 4 Exner G M, Porush J G, Goldstein M R & Levitt M F. An appraisal of free water reabsorption in man. *N Y J Surg Hosp* 29 38 1962
- 5 Goldberg M, McCurdy D K & Ramirez M A. Differences between saline and mannitol diuresis in hydropenic man. *J Clin Invest* 44 182 1965
- 6 Hollwachs O. Klinische Untersuchungen über die Mannitoldiurese an Gesunden und postoperativen Patienten. *Arch Wschr* 43 546 1965
- 7 Hatch F E, Culbertson J W & Diggs L W. Nature of the renal concentrating defect in sickle cell anemia. *J Clin Invest* 46 336 1967
- 8 Miles B E, Paton A & deWardener H E. Minimum urine concentration. *Brit Med J* 11 901 1951
- 9 Perillie P E & Epstein F H. Sickling phenomenon produced by hypertonic solutions. A possible explanation of the hyposthenuria of sickle cells. *J Clin Invest* 42 570 1963
- 10 Porush J B & Abramson R. The effect of hypertonic mannitol on renal sodium reabsorption in hydropenic man. *Clin Sci* 29 475 1965
- 11 Raisz L G, Au W Y W & Scheer R L. Studies on the renal concentrating mechanism IV. Osmotic diuresis. *J Clin Invest* 38 1725 1959
- 12 Rapoport S, Brodsky W A, West C D & MacLer B. Urinary flow and excretion of solutes during osmotic diuresis in hydropenic man. *Amer J Physiol* 156 433 1949
- 13 Scheer R, Quethorst E, Häfner D & Wigger W. Beeinflussung der Nierenfunktion durch Mannitolfusion bei akuter und chronischer Niereninsuffizienz. *Schweiz Med Wschr* 95 11 1133 1965
- 14 Whitten C F & Yonnes A A. A comparative study of renal concentrating ability in children with sickle cell anemia and in normal children. *J Lab Clin Med* 55 400 1960
- 15 Winberg J. Determination of renal concentrating capacity in infants and children without renal disease. *Acta Paediatr Scand* 43 318 1959
- 16 Zik G A, Bruz C & Smith H W. The mechanism of formation of osmotically concentrated urine during the antidiuretic state. *J Clin Invest* 33 1064 1954

Submitted Oct 29 1969

Accepted Febr 12 1970

(H C S) Surgical Department B Rikshospitalet  
Oslo University Hospital  
Oslo  
Norway

**Key words:** Renal concentrating capacity, urinary secretion, mannitol diuresis, osmolar clearance

Table 3 Effect of pentobarbital pretreatment on sodium salicylate induced foetal damage late in pregnancy

Gestation days injected	Substance and dose per 20 g	No of litters	No of foetuses	Foetal mortality		Superficial haemorrhage in living foetuses		Liver haemorrhage in living foetuses		Gastric haemorrhage in living foetuses	
				No	%	No	%	No	%	No	%
15-16	Pentobarbital 1.5 mg										
17	Salicylate 10 mg	20	147	45	31	27	26	9	9	38	37
15-16	Pentobarbital 1.5 mg	10	83	4	5	1	1	0	0	1	1

times. Four of the ten females given 15 mg/20 g died within 24 hrs after injection. Five of the remaining six gave birth before sacrifice. In all, all foetuses were dead.

The effect of single and repeated administration of salicylate was compared and showed that repeated injections did not increase the incidence of foetal damage (Table 2). The injection of 10 mg/20 g salicylate on gestation days 15, 16 and 17 resulted in the death of 47% of the foetuses. When the same dose of salicylate was given on only one of the days 15, 16 or 17 foetal death occurred in 39%, 55% and 61% of the foetuses respectively. However a dose of 3 mg salicylate/20 g on day 15 and 16 followed by 10 mg/20 g on day 17 gave a foetal death incidence of only 21%. The difference in incidence of foetal death between this group and the group given 10 mg/20 g only on day 17 was highly significant ( $p < 0.001$ ). Subcutaneous subcapsular liver and submucosal stomach haemorrhage was found in living foetuses from all groups

except that treated only on day 15 (Table 2). Vessel anomalies of the type described earlier (17) were found after injection on day 15.

Pretreatment with pentobarbital in narcotic doses on gestation day 15 and 16 followed by salicylate treatment of 10 mg/20 g on day 17 also diminished the foetal damaging effect exerted by salicylate given as a single dose (Tables 2 and 3). Pretreatment with pentobarbital resulted in a foetal death rate of 31% compared to 61% when salicylate was given alone. The difference is highly significant ( $p < 0.001$ ). The group receiving only pentobarbital on days 15 and 16 showed almost the same death rate (i.e. 5%) as the un.injected control group (i.e. 1%) (Tables 1 and 3). As in the other experimental groups superficial liver and gastric haemorrhages were found in some living foetuses (Table 3).

Maternal liver microsomal hydroxylating enzyme for aminopyrine demethylation was increased by pentobarbital but not by salicylate administration (Table 4). No change was ob-

Table 4 Effect of drug pretreatment on liver microsomal hydroxylating enzymes in adult mice on day 17 of gestation

No of animals	Pretreatment	Aminopyrine Demethylation nmol/min/mg protein	NADPH-cytochrome b <sub>5</sub> reductase $\mu$ moles NADPH ox./min/mg protein	Cytochrome P-450 $\Delta E$ 450-490 nm/mg protein
		7.85	0.093	0.093
		6.84	0.099	0.011
2	Pentobarbital 1.5 mg/20 g day 15 and 16	14.82	0.066	0.011
	Salicylate 3 mg/20 g day 15 and 16	4.96	0.120	0.012
2	Salicylate 10 mg/20 g day 15 and 16	8.48	0.131	0.012
		7.45	0.118	0.013
		6.78	0.111	0.011
			0.116	0.009



Table 1 Effect of different doses on sodium salicylate induced foetal damage on day 17 of gestation

Dose of salicylate per 20 g	No of litters	No of foetuses	Foetal mortality		Superficial haemorrhage in living foetuses		Liver haemorrhage in living foetuses		Gastric haemorrhage in living foetuses	
			No		No		No		No	
—	10	74	1	1	0	0	0	0	0	0
3 mg	10	76	3	4	0	0	1	1	0	0
10 mg	10	77	54	70	9	39	3	13	5	22
15 mg	10 <sup>a</sup>	9	9	100						

<sup>a</sup> Four females died within 24 hrs after injection. Five delivered before dissection.

(a2) 1.5 mg/20 g on gestation day 15 and 16 followed by a 10 mg/20 g salicylate injection on day 17 of gestation. A group of 10 was given only pentobarbital in the same dosage. The observations of foetal damage were made as described above.

The influence of salicylate and pentobarbital pretreatment on maternal liver microsomal hydroxylase enzymes was studied in 8 animals on day 17 of gestation (Table 4). Two animals were treated with pentobarbital 1.5 mg/20 g, two animals with 3 mg/20 g salicylate and two others with 10 mg/20 g. Two untreated animals of the same gestational age served as controls. Liver microsomes were isolated as described by Ernster *et al.* (11). Protein was measured according to the method of Lowry *et al.* (20). The oxidative demethylation of aminopyrine and NADPH-cytochrome c reductase as well as the amount of cytochrome P 450 in the liver microsomes were assayed as previously described (6, 23).

The author is gratefully indebted to Dr Sten Orrenius and his staff for helping with this part of the study.

## RESULTS

LD 50 for sodium salicylate in adult virgin females of the A/Jax strain was found to be 15.2 mg/20 g body weight when given as a single i.m. injection in 0.1 ml distilled water. Confidence limits 14.4–16.2 (at  $p=0.05$ ).

The study of the effect of different doses of salicylate on the foetus late in pregnancy showed that 3 mg/20 g produced little or no foetal damage (Table 1). After the injection of 10 mg/20 g foetal death was found in 70%. Subcutaneous and subcapsular liver haemorrhages of a type described earlier (9) were found in living foetuses at incidences of 39% and 13% respectively. Moreover macroscopically visible submucosal haemorrhages of the stomach was seen in 22% of the fo-

Table 2 Effect of single and repeated administration on sodium salicylate induced foetal damage late in pregnancy

Gestation days injected	Dose of salicylate per 20 g	No of litters	No of foetuses	Foetal mortality		Superficial haemorrhage in living foetuses		Liver haemorrhage in living foetuses		Gastric haemorrhage in living foetuses		Vessel anomalies in living foetuses	
				No		No		No		No		No	
15	10 mg	10	74	29	39	1	2	0	0	0	0	12	27
16	10 mg	10 <sup>a</sup>	58	32	55	11	42	0	0	0	0	0	0
17	10 mg	20 <sup>b,d</sup>	134	82	61	14	27	6	12	16	31	0	0
15, 16, 17	10 mg	20	152	72	47	3	4	4	5	7	9	26	33
15, 16	3 mg												
17	10 mg	20	140	32	21	4	3	14	14	43	36	0	0

<sup>a</sup> 1 delivered before dissection.

<sup>b</sup> 2 delivered before dissection.

<sup>c</sup> 1 delivered before dissection.

<sup>d</sup> 10 of these are the same ones as in the dose experiment.

## SUMMARY

The LD 50 for sodium salicylate in adult A/Jax mice has been determined and was found to be 15.2 mg/20 g. Three different doses (15 mg/10 mg and 3 mg/20 g) have been tested for their foetal damaging effect on day 17 of gestation. The 15 mg dose produced maternal and foetal death or premature delivery. Different types of foetal damage such as foetal death and foetal haemorrhages were found with the 10 mg dose. The 3 mg dose had little or no effect on the foetus.

Sodium salicylate in a dose of 10 mg/20 g was given on days 15, 16 and 17 and compared to a group given salicylate only on day 17. Foetal death was reduced in the group given repeated injections. Sodium salicylate in a dose of 3 mg/20 g was given on days 15 and 16 followed by 10 mg/20 g on day 17. Compared to the group given salicylate 10 mg/20 g only on day 17 there was a highly significant decrease in foetal mortality. Pentobarbital in a narcotic dose of 1.5 mg/20 g was given on day 15 and 16 of gestation followed by salicylate 10 mg/20 g on day 17. Foetal mortality was significantly reduced when compared with the group treated with a single salicylate injection of 10 mg/20 g on day 17 of gestation.

Induction of hydroxylating enzymes was studied in a few animals. In the adult day 17 pregnant female pentobarbital but not salicylate treatment gave increased enzyme activity.

The results are discussed with reference to the design of teratology tests. Furthermore the mechanisms behind the modifying effect of repeated drug administration are debated.

## ACKNOWLEDGEMENTS

I take this opportunity of expressing my sincere gratitude to Drs K. Soos-Larsson and Sten Örnemark for valuable support during the investigation and Sören J. Yaffe for critical reading of the manuscript. I also wish to thank Mrs Tofine Palm and Mrs Anne Marie Johansson for skilful secretarial and technical assistance.

The expenses of the investigation were defrayed by grants from the Swedish Medical Research Coun-

cil (14X 993 05 and 14P 993-05) from Expressens presattfond from the Association for the Aid of Crippled Children New York U.S.A. and from the Swedish Foundation Theres och Johan Anderssons Minne.

## REFERENCES

1. Ames B. M., Gartner L., Forman M. & Wolfson S. Effect of several drugs and chemicals on hepatic glucuronide formation in newborn rats. *Proc Soc Exp Biol Med* 112 1037 1963.
2. Baker J. B. E. The effects of drugs on the foetus. *Pharmacol Rev* 12 37 1960.
3. Burns J. J., Cucinell S. A., Koester R. & Conney A. H. Application of drug metabolism to drug toxicity studies. *Ann N Y Acad Sci* 123 273 1965.
4. Ostr, C. & Yaffe S. J. Barbiturate enhancement of bilirubin conjugation and excretion in young and adult animals. *Prober Res* 2 361 1968.
5. Cooney A. H. Pharmacological asphixiation of microsomal enzyme induction. *Pharmacol Rev* 19 317 1967.
6. Dallner G. Studies on the structural and enzymic organization of the membranous elements of liver microsomes. *Acta Path Microbiol Scand Suppl* 166 1963.
7. Done A. K. Perinatal pharmacology. *Ann Rev Pharmacol* 6 189 1966.
8. Eriksson M. Effect of salicylate treatment on foetal and maternal prothrombin time in the mouse. *Acta Physiol Scand* 78 38 1970.
9. — Salicylate induced foetal haemorrhages in two mouse strains. *Acta Path Microbiol Scand* 76 164 1969.
10. Eriksson M. & Larsson K. S. Premature birth induced in mice by salicylate. *Nature* 220 385 1968.
11. Ernster L., Sakschitz, P. & Palade G. E. Enzyme-structure relationships in the endoplasmic reticulum of rat liver. *J Cell Biol* 15 541 1962.
12. Franke H. & Klinger W. Untersuchungen zum Mechanismus der Enzyminduktion IX. Die Wirkung von Barbitat auf die Substratkin der Leberzellen grävender Ratten und ihrer Foeten. *Acta Biol Med German* 17 507 1966.
13. Guidelines for reproduction studies for safety evaluation of drugs for human use. FDA USA 1966.
14. Hart L. G., Adamson R. H., Dotson, R. L. & Fouts, J. R. Stimulation of hepatic microsomal drug metabolism in the newborn and fetal rabbit. *J Pharmacol Exp Ther* 137 103 1962.
15. Hammarén O. Effect of salicylamide administration on D-glucuronic acid metabolism in the rat. *Ann Acad Sci Fenn (Med)* 123 1 1966.
16. Larsson K. S. Studies on the closure of the secondary palate. III. Autoradiographic and histochemical studies in the normal mouse embryo. *Acta Morph Anat Scand* 4 349 1962.
17. Larsson K. S., Bostrom H. & Eriksson B. Sal-

served for NADPH-cytochrome c reductase or cytochrome P-450. The values are calculated in relation to the protein content.

## DISCUSSION

The most interesting finding in this investigation is the protection against the foetal damaging effect of a single high dose of salicylate by pretreatment with small doses of salicylate. It is not known if salicylate pretreatment acts via the mother, the foetus or both. Certain drugs, even in small doses, have been shown to increase the level of liver microsomal enzymes with a concomitant faster detoxification of drugs (5). Human newborns of phenobarbital-treated mothers have greater capacity to conjugate bilirubin (21). Moreover, in animal experiments, phenobarbital and benzpyrene have been shown to increase the activity of liver enzymes in the foetus and newborn when given to the mother late in pregnancy (1, 4, 14). This observation has not been substantiated in morphological studies of smooth surfaced endoplasmic reticulum (12). However, this induction of enzyme activity can only take place in the foetus during the very last days of gestation (14).

Barbituric acid derivatives are the most common inducers of enzyme activity (4, 12). In this study, pretreatment with pentobarbital had about the same protective effect against foetal damage as pretreatment with small doses of salicylate. This suggests that drug-induced enhancement of detoxification of salicylate could be one explanation for the protective effect of both pentobarbital and salicylate. The results from the biochemical studies on liver microsomal enzyme induction do not support this assumption. However, the activities investigated in the present study did not include glucuronide and glycine formation, which are the most important pathways of salicylate detoxification (25, 26). There are diverging reports in the literature on the effect of salicylate on its own metabolism. On the one hand re-

peated salicylamide administration to rats has been shown to decrease its toxicity and increase the urinary output of glucuronic acid (15). On the other hand, chronic administration of acetylsalicylic acid to dogs does not cause a decrease in its serum concentration (3).

The current recommendations for reproductive studies for safety evaluation of drugs for human use now include perinatal studies (13, 24). Concerning the doses and the method of administration, there are no differences from the studies in the organogenetic period. Thus, it is recommended to use at least two doses which should be selected with reference to the dose response of the adult animal. In this study, 15 mg/20 g, a dose close to LD<sub>50</sub> (15.2 mg/20 g) was chosen for comparison to the empirically found teratogenic dose of 10 mg/20 g (17). The lethal effect of the high dose in pregnant animals was of the same magnitude as in non-pregnant females. Premature birth has been regarded as an expression of foetal damage of salicylate treatment late in pregnancy (10). This was also found in the present study but only with the highest dose used. Other types of foetal damage, such as haemorrhages and death, were found with the 10 mg dose chosen because of its empirically established teratogenic effect (17). It is interesting to note that there is a very small range between the LD<sub>50</sub> for adult mice and the potent foetal damaging dose. Studies on tumour-inhibiting chemicals have been made earlier in which LD<sub>50</sub> and the teratogenic dose were compared and the range was shown to vary considerably (22).

In teratological experiments, drugs are sometimes given on only one day of gestation but more commonly administered throughout pregnancy. This study has shown, however, that continuous administration of a drug, even for a short period, is able to modify the teratogenic response.

The results of the present investigation support the need for further pharmacological studies in the safety evaluation of drugs.

## SUMMARY

LD 50 for sodium salicylate in adult /Jax mice has been determined and was found to be 15.2 mg/20 g. Three different doses (15 mg, 10 mg and 3 mg/20 g) have been tested for their foetal damaging effect on day 17 of gestation. The 15 mg dose produced maternal and foetal death or premature delivery. Different types of foetal damage such as foetal death and foetal haemorrhages were found with the 10 mg dose. The 3 mg dose had little or no effect on the foetus.

Sodium salicylate in a dose of 10 mg/20 g was given on days 15, 16 and 17 and compared to a group given salicylate only on day 17. Foetal death was reduced in the group given repeated injections. Sodium salicylate in a dose of 3 mg/20 g was given on days 15 and 16 followed by 10 mg/20 g on day 17. Compared to the group given salicylate 10 mg/20 g only on day 17 there was a highly significant decrease in foetal mortality. Pentobarbital in a narcotic dose of 1.5 mg/20 g was given on day 15 and 16 of gestation followed by salicylate 10 mg/20 g on day 17. Foetal mortality was significantly reduced when compared with the group treated with a single salicylate injection of 10 mg/20 g on day 17 of gestation.

Induction of hydroxylating enzymes was studied in a few animals. In the adult day 17 pregnant female pentobarbital but not salicylate treatment gave increased enzyme activity.

The results are discussed with reference to the design of teratology tests. Furthermore the mechanisms behind the modifying effect of repeated drug administration are debated.

## ACKNOWLEDGEMENTS

I take this opportunity of expressing my sincere gratitude to Dr A. Sören Larsson and Sten Örrman for valuable support during the investigation and Sören J. Yaffe for critical reading of the manuscript. I also wish to thank Mrs Yellan Palm and Mrs Anne Marie Johansson for skilful secretarial and technical assistance.

The expenses of the investigation were defrayed by grants from the Swedish Medical Research Coun-

cil (140X 993-05 and 14P 993 05) from Expressens prenatalfond from the Association for the Aid of Crippled Children New York U.S.A. and from the Swedish Foundation Therese och Johan Anderssons Minne.

## REFERENCES

- 1 Arias I M, Gartner L, Farman M & Wotfson J. Effect of several drugs and chemicals on hepatic glucuronide formation in newborn rats. *Proc Soc Exp Biol Med* 112 1037 1963.
- 2 Baker J B E. The effects of drugs on the foetus. *Pharmacol Rev* 12 37 1960.
- 3 Burns J J, Cocanelli S A, Koster R & Conney A H. Application of drug metabolism to drug toxicity studies. *Ann NY Acad Sci* 123 273 1965.
- 4 Catz C & Yaffe S J. Barbiturate enhancement of bilirubin conjugation and excretion in young and adult animals. *Pediatr Res* 2 361 1968.
- 5 Conney A H. Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev* 19 317 1967.
- 6 Dallner G. Studies on the structural and enzymic organization of the membranous elements of liver microsomes. *Acta Path Microbiol Scand Suppl* 166 1963.
- 7 Dose A. K. Perinatal pharmacology. *Ann Rev Pharmacol* 6 189 1966.
- 8 Eriksson M. Effect of salicylate treatment on fetal and maternal prothrombin time in the mouse. *Acta Physiol Scand* 78 39 1970.
- 9 — Salicylate induced foetal haemorrhage in two mouse strains. *Acta Path Microbiol Scand* 76 164 1969.
- 10 Eriksson M & Larsson A. S. Premature birth induced in mice by salicylate. *Nature* 220 385 1968.
- 11 Ermer L, Sekevertz P & Palade O E. Enzyme-structure relationships in the endoplasmic reticulum of rat liver. *J Cell Biol* 15 541 1962.
- 12 Franke H & Winger W. Untersuchungen zum Mechanismus der Enzyminduktion IX. Die Wirkung von Barbitol auf die Substruktur der Leber zellen grosser Ratten und ihrer Foeten. *Acta Biol Med German* 17 507 1966.
- 13 Guidelines for reproduction studies for safety evaluation of drugs for human use. FDA USA 1966.
- 14 Hart L III, Adamson R H, Dixon R L & Fouts J R. Stimulation of hepatic microsomal drug metabolism in the newborn and fetal rabbit. *J Pharmacol Exp Ther* 137 103 1962.
- 15 Hammett O. Effect of salicylamide administration on D-glucuronic acid metabolism in the rat. *Ann Acad Sci Fenn (Med)* 13 1 1966.
- 16 Larsson A. S. Studies on the closure of the secondary palate III. Autoradiographic and histochemical studies in the normal mouse embryo. *Acta Morph Neerl Scand* 4 349 1966.
- 17 Larsson A. S., Bostrom H & Ericson, B. Sal-

- cylate induced malformations in mouse embryos  
*Acta Paediat Scand* 52 36 1963
- 18 Larsson L S & Eriksson M Salicylate induced fetal death and malformations in two mouse strains *Acta Paediat Scand* 55 569 1966
  - 19 Litchfield J T & Wilcoxon F A simplified method of evaluating dose-effect experiments *J Pharmacol Exp Ther* 96 99 1949
  - 20 Lowry O H Rousebrough N J Farr A L & Randall R J Protein measurement with the Folin phenol reagent *J Biol Chem* 193 265 1951
  - 21 Maurer H M Wolff J A Finster M Poppers P J Pintuck E Kuntzman R & Conney A H Reduction in concentration of total serum bilirubin in offspring of women treated with phenobarbitone during pregnancy *Lancet* II 122 1968
  - 22 Murphy M L Teratogenic effects of tumour inhibiting chemicals in the foetal rat in G E W Wolstenholme & C M O'Connor (eds) *Ciba Foundation Symposium on Congenital Malformations* J & A Churchill London 1960 p 78
  - 23 Orrenius S & Thor H Fatty acid interaction with the hydroxylating enzyme system of rat liver microsomes *Europ J Biochem* 9 415 1969
  - 24 Report of a WHO Scientific Group Principles for the testing of drugs for teratogenicity *Pharm Ind* 29 759 1967
  - 25 Smith M J H & Smith P K. *The Salicylates* John Wiley & Sons New York 1966
  - 26 Sturman J A Dawkins P D McArthur N & Smith M J H The distribution of salicylate in mouse tissues after intraperitoneal injection. *J Pharm Pharmacol* 20 58 1968
  - 27 Tuchmann-Duplessis H Design and interpretation of teratogenic tests in J M Robson J Sullivan & R L Smith (eds) *Biological Cross Symposium on Embryopathic Activity of Drugs* J & A Churchill London 1965 p 56

Submitted Jan 24 1970

Accepted Febr 17 1970

Laboratory of Teratology

Karolinska Institutet

S-104 01 Stockholm

Sweden

**Key words** Prenatal influences abnormalities drug induced sodium salicylate

## DIAGNOSIS OF BETA THALASSAEMIA IN THE NEWBORN BY MEANS OF HAEMOGLOBIN SYNTHESIS

DI GABURRO S VOLPATO and V VIGI

From the Clinica Pediatrica (Head D Gaburro)  
University of Ferrara Ferrara Italy

It is now well established that the most significant metabolic defect in beta Thalassemia is a decreased ability of erythroid cells to synthesize beta globin chains resulting in a defective production of Hb A with a relative excess of alpha chains synthesis. The defect in beta chains synthesis can be partial (2-7-12) or complete (3-5-10).

These abnormalities can be detected in the erythroid cells from both peripheral blood (2-3-7-10-12) and bone marrow (5). A prominent role in the genesis of the thalassaemic anemia has been ascribed to the excess in alpha chains synthesis (2) and a strict correlation between the rate of excess alpha chain synthesis and the reduced survival of thalassaemic erythrocytes labelled with radioactive chromate and transfused into healthy group compatible recipients has been demonstrated (10).

Since the main metabolic defect in beta Thalassemia is genetically determined it should be present at birth because both normal newborn and premature infants synthesize constant amounts of beta globin chains. The demonstration at birth of a disturbance in haemoglobin synthesis due to defective beta chains production would enable early diagnosis of beta Thalassemia.

Such early detection would be important not only in relation to the possibility of prompt

supportive therapy or a future specific treatment of the disease but also permit studies of the intrinsic metabolic defect before the onset of secondary factors. It would also enable a description of the development of secondary factors which contribute to create the clinical pattern observed in these patients at a later stage of the disease.

With this purpose in mind we have carried out a study of haemoglobin synthesis in three newborns whose parents are carriers of the beta Thalassemia trait.

### SUBJECTS AND METHODS

#### *Subjects studied*

Three newborns (cases 1-2 and 3) whose parents are heterozygotes for the beta Thalassemia gene have been studied. At 3 days and (in cases 1 and 2) at 2-4 and 5 months of age the following investigations were performed: haemoglobin synthesis, haemoglobin electrophoresis both by starch gel and cellulose acetate membrane, determination of alkali resistant haemoglobin, red blood cell osmotic resistance and complete blood counts.

A control study of haemoglobin synthesis was performed in two premature newborns (cases 4 and 5) whose parents had no haematological abnormalities and in two children aged 2 and 7 years (cases 6 and 7) with reticulocytosis secondary to bleeding an anemia.

#### *Clinical data*

*Case 1 (C. Z.)* Born at term. Parents heterozygotes for beta Thalassemia gene. Elder brother affected by beta Thalassemia major.

The pregnancy and the delivery were uneventful.

Table 1 Haematological data of cases 1, 2 and 3

Case	Age	Hb (g)	RBC (mm <sup>3</sup> )	Erythroblasts (%)	Reticulocytes (%)	Hb A <sub>2</sub> (%)	Alkali resistant Hb (%)	Osmotic fragility test NaCl		
								Min	Med	Max
1 C Z	3 days	15.8	5 150 000	4	30	—	94	0.46	0.34	0.21
	2 mo	12.8	4 310 000	0	29	1	58	0.46	0.32	0.20
	4 mo	12.5	4 100 000	0	25	1.6	30	0.45	0.31	0.20
	5 mo	12.0	3 800 000	0	15	2	—	0.44	0.34	0.16
2 S S	3 days	16.0	5 300 000	3	35	—	100	0.48	0.34	0.20
	2 mo	12.6	4 290 000	2	49	1.7	98	0.45	0.32	0.16
	4 mo	9.6	3 650 000	2	50	2.5	98	0.45	0.31	0.26
	5 mo	6.0	2 600 000	9	50	2.6	97	0.44	0.30	0.24
3 S C	2 days	15.2	5 050 000	5	32	—	100	0.48	0.33	0.19
	2 mo	4.0	1 800 000	10	60	2.2	97	0.44	0.30	0.23

Birth weight was 2750 g. He has always been in good health. When last examined (12 months) the clinical and haematological examinations were in the normal range.

**Case 2 (S S)** Born at term. Parents bear the beta Thalassemia trait. Birth weight was 2700 g. The clinical examination at the third day of life was normal.

She remained well and the growth and development continued normally till the fifth month when hypochromic anaemia and splenomegaly were detected. The clinical and haematological pattern of beta Thalassemia major is now clear and transfusion treatment has been started.

**Case 3 (S C)** Born at term. Parents are heterozygotes for the beta Thalassemia gene. Three siblings are affected by beta Thalassemia major and one is normal. The pregnancy and the delivery were uneventful. Birth weight was 3300 g. Neonatal examination revealed no clinical signs except mild jaundice which became evident on the third day of life and disappeared in a few days.

She remained well until the second month of life.

when hypochromic anaemia and hepatomegaly without splenomegaly was noted. At this age clinical observation and haematological laboratory data were typical for beta Thalassemia major.

**Cases 4 and 5** were prematurely born after uneventful pregnancies of 33 and 31 weeks respectively. At the present time they are 14 and 24 months old and in good health. The parents bear no signs of beta Thalassemia or other haematological abnormalities.

**Cases 6 and 7** suffered from mild bleeding tendencies and were selected because of the high reticulocyte count found in the peripheral blood. The parents are not carriers of the beta Thalassemia trait and presented no other haematological abnormalities.

The haematological data of subjects 1, 2 and 3 are summarized in Table 1.

#### Methods

Routine haematological studies were performed according to the techniques described by Dacie (6).

Fetal haemoglobin percentages were determined by the alkali denaturation method of Singer et al (7).

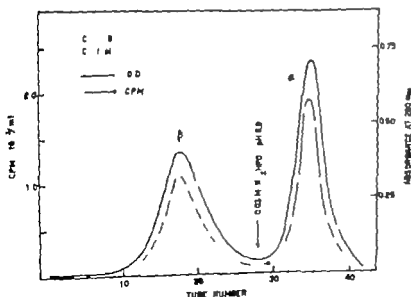


Fig. 1 Elution pattern from CM on column chromatography of <sup>125</sup>I-labelled globin chains of case 6 (bleeding anaemia) after incubation of the erythrocytes with tritiated amino acids.

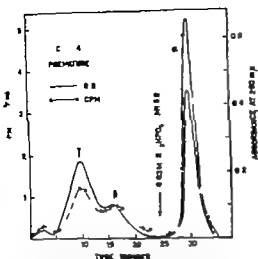


Fig 2 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 4 (premature) after incubation of the erythrocytes with tritiated amino acids

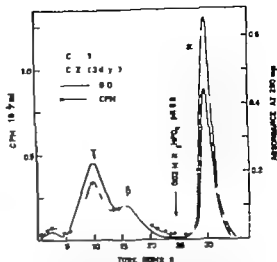


Fig 3 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 1 (C Z) after incubation of the erythrocytes with tritiated amino acids

Starch gel and cellulose acetate membrane electrophoreses were performed using the methods described by Huisman (8) and Brune (4) respectively.

Haemoglobin synthesis was studied by incubating samples of the peripheral blood with all the amino-acids required for protein synthesis; valine, leucine, lysine were tritiated. Globins prepared by the acid acetone method were subjected to column chromatography on CM cellulose and the eluted peaks conventionally quantitated with a Gelford automatic record and spectrophotometer; the radioactivity incorporated was directly counted in a Tri-carb liquid scintillation counter. The detailed technical procedures have been described elsewhere (3) and were followed without

modifications. Haemoglobin column chromatography on Amberlite IRC 50 was performed following the indications of Allen *et al* (1). All blood samples used for study were collected by venipuncture in heparinized syringes from fasting subjects and used immediately.

## RESULTS

In the two control subjects (cases 6 and 7) haemoglobin synthesis was normal. The elution profile after chromatography of the labelled

Table 2 [ $^3\text{H}$ ] amino acid (leucine, valine, lysine) incorporation into  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  globin chains in three newborns whose parents are heterozygotes for the  $\beta$  thalassaemia gene

Case	Age	Radioactivity*			
		[ $^3\text{H}$ ] $\alpha$	[ $^3\text{H}$ ] ( $\beta + \gamma + \delta$ )		$\frac{[\text{H}] \alpha}{[\text{H}] (\beta + \gamma + \delta)}$
1 C Z "healthy"	3 days	9 730	9 600		1.01
	2 mo	8 500	8 500		1.00
	4 mo	6 100	6 200		0.99
	5 mo	5 700	5 800		0.99
		[ $^3\text{H}$ ] $\alpha$	[ $^3\text{H}$ ] $\beta$	[ $^3\text{H}$ ] ( $\gamma + \delta$ )	$\frac{[\text{H}] \alpha}{[\text{H}] (\gamma + \delta)}$
2 S S Thalassaemia major	3 days	49 000	0	24 300	2.0
	2 mo	78 300	0	41 000	1.9
	4 mo	41 500	0	21 800	1.9
	5 mo	48 900	0	25 400	1.9
	2 days	70 700	0	39 000	1.8

\*Radioactivity is expressed as counts/min incorporated into 0.4 ml of packed red cells



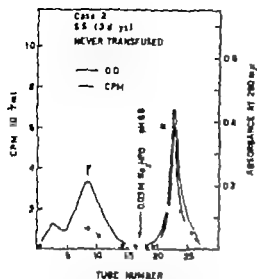


Fig. 4 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 2 (S S) after incubation of the erythrocytes with labelled amino acids

globin chains on CM (Fig. 1) reveals the presence of two peaks of proteins corresponding to the beta and alpha globin chains. Both peaks were radioactive indicating active synthesis of beta and alpha globin chains. The same amount of protein and incorporated radioactivity was present under the two peaks, as found in normal adult subjects ratio  $\alpha/\beta + \delta = 1$ . In the two premature infants (cases 4 and 5) the elution pattern after CM chromatography revealed three radioactive protein peaks corresponding to gamma, beta and alpha globin chains (Fig. 2). The ratio  $\alpha/\gamma + \beta + \delta$  is equal to 1 both for radioactivity and for protein. The high specific activity of the beta chain demonstrates that the change from gamma to beta chain production is under way.

In case 1 (C Z) haemoglobin synthesis studied on the third day of life was normal: the elution pattern (Fig. 3) being identical to that found in the normal newborns and pretermatures. Haemoglobin synthesis studied at the

third and fifth months of life showed a gradual substitution of gamma chains by beta chains (Table 2).

In cases 2 and 3 (S S and S C) haemoglobin synthesis studied at birth presented an abnormal chromatographic pattern: the elution profile shows complete absence of the peak corresponding to beta globin and an excess synthesis of alpha globin chains over the other globin chains (Figs 4 and 5 and Table 2) resulting in an abnormal  $[H] \alpha/[H] (\gamma + \delta)$  characteristic of beta thalassaemia major. Chromatography on Amberlite IRC 50 (Fig. 6) revealed the presence of only haemoglobin F and confirmed the results.

## DISCUSSION

The clinical course and changes in the haematological data during followup examinations (Table 1) confirmed the results obtained by means of haemoglobin synthesis at birth. Case 1 who presented a normal haemoglobin synthesis at birth did not develop any subsequent haematological abnormalities and was healthy at examination at the age of 18 months. In cases 2 and 3 who manifested disorders in haemoglobin synthesis at birth characterized by an absence of beta globin chain synthesis

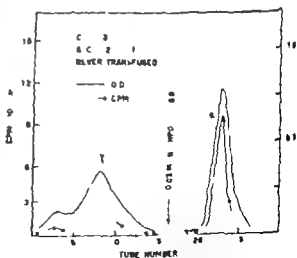


Fig. 5 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 3 (S C) after incubation of the erythrocytes with labelled amino acids

<sup>1</sup> Delta chain cannot be separated with this chromatographic technique. Delta chain from purified Hb A<sub>2</sub> is eluted immediately after the beta chain.

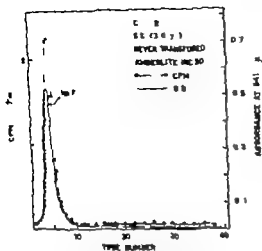


Fig. 4. Elution pattern from Amberlite (IRC 50) of the labeled haemoglobin of case 2 (S.S.) after incubation of the erythrocytes with tritiated amino acids.

and an excess of alpha globin chain synthesis beta Thalassaemia major has become clinically evident during followup.

These findings clearly indicate that the diagnosis of beta Thalassaemia can be confirmed at birth when typical clinical features are absent by demonstrating the existence of the specific metabolic defect of the thalassaemic red cell i.e. absence of beta globin chain synthesis and excess of alpha globin chain synthesis. This can be demonstrated even when other routine haematological studies are normal.

This method permits also a neonatal diagnosis of the beta Thalassaemia trait as Wai Han & Nathan have recently demonstrated (11).

It must also be pointed out that if we consider the patterns of haemoglobin synthesis in the premature, this possibility must be extended also to the prenatal period (at least from the seventh month of intrauterine life).

### SUMMARY

Haemoglobin synthesis has been studied in three newborns whose parents are carriers of the beta Thalassaemia trait. Demonstration of absence of beta globin chain synthesis and ex-

cess of alpha globin chain synthesis permitted diagnosis of beta Thalassaemia major in two of three subjects in absence of any other sign of the disease.

The clinical course and change in the haematological data during followup examinations confirmed the diagnosis performed at birth. In the third subject no disorder in haemoglobin synthesis was ascertained at birth; he remained well and is now perfectly healthy. On this evidence it is suggested that beta Thalassaemia can be detected at birth in absence of any other sign of the disease.

### REFERENCES

- Allen D. W., Schroeder W. A. & Balog J. Observations on the chromatographic heterogeneity of normal adult and fetal human haemoglobin: a study on the effects of crystallization and chromatography on the heterogeneity and isoelectric content. *J. Amer. Chem. Soc.* 80: 1629 1958.
- Bank A. & Marks P. A. Excess  $\alpha$ -chain synthesis relative to  $\beta$ -chain synthesis in Thalassaemia major and minor. *Nature* 212: 1198 1966.
- Bargellesi A., Pontremoli S. & Conconi F. Absence of  $\beta$  globin synthesis and excess of  $\alpha$  globin synthesis in homozygous  $\beta$  Thalassaemia. *Europ. J. Biochem.* 1: 73 1967.
- Brace R. O., Golias T. & Balakas J. G. Rapid qualitative and quantitative hemoglobin fractionation. *Amer. J. Clin. Path.* 44: 695 1965.
- Conconi F., Bargellesi A., Pontremoli S., Vignani V., Volpato E. & Gabrillo D. Absence of  $\beta$  globin synthesis and excess of  $\alpha$  globin synthesis in homozygous  $\beta$  Thalassaemic subjects from the Ferrara Region. *Nature* 217: 259 1968.
- Craciun I. V. *Metabolic Diseases of the Blood*. Padova 1957.
- Haywood D., Karow M. & Wenzman S. Asymmetrical incorporation of monomethyl in the alpha and beta chains of hemoglobins synthesized by thalassaemic reticulocytes. *J. Lab. Clin. Med.* 66: 46 1965.
- Husman T. H. J. Normal and abnormal human hemoglobins. *Advances Clin. Chem.* 6: 476 1963.
- Sanger R., Chernoff A. L. & Sanger L. Studies on abnormal hemoglobins: their demonstration in sickle cell anemia and other hemoglobin disorders by means of alkali denaturation. *Blood* 6: 413 1951.
- Vignani V., Volpato E., Gabrillo D., Conconi F., Bargellesi A. & Pontremoli S. The correlation between red cell survival and excess of  $\alpha$  globin

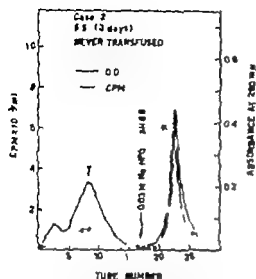


Fig. 4 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 2 (S S) after incubation of the erythrocytes with tritiated amino acids

globin chains on CM (Fig. 1) reveals the presence of two peaks of proteins corresponding to the beta and alpha globin chains. Both peaks were radioactive indicating active synthesis of beta and alpha globin chains. The same amount of protein and incorporated radioactivity was present under the two peaks as found in normal adult subjects: ratio  $\alpha/\beta + \delta = 1$ . In the two premature infants (cases 4 and 5) the elution pattern after CM chromatography revealed three radioactive protein peaks corresponding to gamma, beta and alpha globin chains (Fig. 2). The ratio  $\alpha/\gamma + \beta + \delta$  is equal to 1 both for radioactivity and for protein. The high specific activity of the beta chain demonstrates that the change from gamma to beta chain production is under way.

In case 1 (C Z) haemoglobin synthesis studied on the third day of life was normal, the elution pattern (Fig. 3) being identical to that found in the normal newborns and pretermatures. Haemoglobin synthesis studied at the

third and fifth months of life showed a gradual substitution of gamma chains by beta chains (Table 2).

In cases 2 and 3 (S S and S C) haemoglobin synthesis studied at birth presented an abnormal chromatographic pattern: the elution profile shows complete absence of the peak corresponding to beta globin and an excess synthesis of alpha globin chains over the other globin chains (Figs 4 and 5 and Table 2) resulting in an abnormal  $[\text{H}] \alpha/[\text{H}] (\gamma + \delta)$  characteristic of beta thalassaemia major. Chromatography on Amberlite IRC 50 (Fig. 6) revealed the presence of only haemoglobin F and confirmed the results.

## DISCUSSION

The clinical course and changes in the haematological data during follow-up examinations (Table 1) confirmed the results obtained by means of haemoglobin synthesis at birth. Case 1 who presented a normal haemoglobin synthesis at birth did not develop any subsequent haematological abnormalities and was healthy at examination at the age of 18 months. In cases 2 and 3 who manifested disorders in haemoglobin synthesis at birth characterized by an absence of beta globin chain synthesis

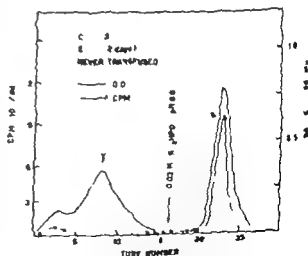


Fig. 5 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 3 (S C) after incubation of the erythrocytes with tritiated amino acids

<sup>1</sup> Delta chain cannot be separated with this chromatographic technique. Delta chain from purified Hb A<sub>2</sub> is eluted immediately after the beta chain.

## GENETIC STUDIES IN GLYCOGEN STORAGE DISEASE TYPE III

PER ERIK WAALER, ODDVARD GARATUN, TIELISTO and PETER JOHAN MOE

*From the Department of Paediatrics and the Department of Biochemistry  
University of Bergen, Bergen, Norway*

Recent biochemical advances have resulted in a division of glycogen storage disease (GSD) into at least 7 types with different enzymatic defects (3, 7, 12, 20). It is now also possible to detect mild forms of the disease. Type III is of particular interest for genetic studies since a deficiency of the debranching enzyme activity usually can be demonstrated in the blood corpuscles. The debranching enzyme system catalyzes the degradation of branching points in glycogen (6).

It is still difficult to obtain definite genetic conclusions in the different types of GSD from the available data in the literature (12). Valuable information was obtained from screening studies of enzyme activity and glycogen content of blood corpuscles from relatives of patients with types III and VI (2, 14, 17, 18, 19). Recently Van Hooft (15) demonstrated that the enzymatic defect of erythrocytes of the parents of 14 patients with GSD type III were in most cases intermediary between the normal and pathological situations. There was however an important overlapping both with normal and pathological values. Moreover Huijing *et al.* (6) when studying the activity of the debranching enzyme system in leucocytes were not able to discover heterozygotes among relatives of patients with GSD type III. It appears therefore that further investigations of the rela-

tives of patients with GSD type III were of great interest. The present paper reports the results of erythrocyte studies performed on samples from a large number of members of four families with this particular type of GSD.

## MATERIALS

The four families with type III have been selected from a total of 20 cases of GSD traced in Norway (Table 1). The 20 cases were collected by contacting different departments at the University Hospitals in Norway and by sending an inquiry to all departments of paediatrics and internal medicine in Norway. Seven of the cases are from the Department of Paediatrics, University of Bergen. Further details will be given in a later publication (Moe *et al.*). Ten of the cases have been typed as to their specific enzyme deficiency. Six of the cases turned out to be of type III. These 6 cases as well as 2 cases of type V and 1 case of type VI are from the Western Coast of Norway.

Amplio-1-4-glucosidase activity and glycogen content were studied in the erythrocytes from 5 of the

Table 1. Cases of glycogen storage disease traced in Norway

GSD type	No of cases	Reported previously	
		No of cases	Author
II	1	1	Klinge (9)
III	6	1	Heyer & Moe (7)
V	2	2	Skjelderud (13)
VI	2	1	Heyer & Moe (7)
Unclassified*	9	0	
Total	20	5	

\* Enzyme studies not performed

The biochemical part of this work was carried out by O. G. T. at the Department of Biochemistry, Arstad 101 in Bergen, Norway.

- synthesis in  $\beta$  Thalassemia *Brit J Haemat* 16  
25 1969
- 11 Wai Kun Y & Nathan D G Beta Thalassemia  
Trait Detection at Birth, *Science* 161 389 1968
- 12 Weatherall D J Clegg J B & Naughton  
M A Globin synthesis in Thalassemia: an in  
vitro study *Nature* 208 1061 1965

Clinica Pediatrica  
Universita di Ferrara  
44100 Ferrara  
Italy

**Key words** Beta thalassemia, newborn haemoph-  
bin synthesis, alpha globin chain synthesis, beta globin  
chain synthesis

Submitted Oct 21 1969

Accepted Febr 20 1970

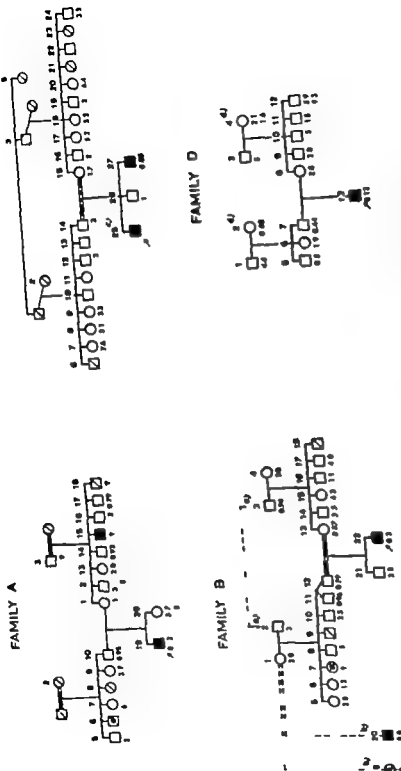


Fig. 1. Pedigrees of 4 families with glycogen storage

Consanguineous marriage

- a) Persons B<sub>2</sub> and the mother of person B<sub>3</sub> are second and third cousins through different lines
- b) Persons B<sub>10</sub> and B<sub>11</sub> are third cousins to person B<sub>2</sub> and to each other. These three persons are also more distantly related to each other in a complicated manner
- c) Specimen from liver examined by professor Hers & de Louvain
- d) Distantly related to person A<sub>11</sub>

Studied relatives. Figure below represents amino 1.6 glucose activity (units/g hemoglobin) of erythrocytes.

Patent with glycogen storage disease type III

Not studied relatives

Probable glycogenosis. Not studied

Stillborn

Died in infancy

Male

Female

Shaded square

Shaded circle

Circle with cross

Open square

Open circle

Open square with cross

Open circle with cross

Table 2 Activity of amylol 1,6 glucosidase in erythrocytes from patients of type III GSD their relatives and healthy controls

Group	No of cases	Range (units/g Hb)	Mean (units/g Hb)
Patients type III	5	0-0.2	0.097
Parents of patients	8	0.79-2.8	1.25
Grandparents of patients	9	0.62-5.9	2.77
Siblings of patients	37	0.5-7.6	3.06
Siblings of patients	3	1.6-5.7	3.36
Healthy controls	15	1.7-7.3	4.10

patients with type III GSD and a total of 57 of the relatives of the patients. The control material consisted of 15 healthy individuals above the age of 10 years. Only slightly higher amylol 1,6 glucosidase activity of erythrocytes was found by Van Hoof (15) in children below 10 years compared to healthy adults. It was therefore considered unnecessary by us to include small healthy children in the control material particularly due to the fact that only 7 of the relatives of the patients were under the age of 10 years. (U/C) glucose was purchased from the Radiochemical Centre Amersham Bucks.

Glycogen type II (from shell fish) was obtained from Sigma.

## DESCRIPTION OF SIX PATIENTS WITH ESTABLISHED TYPE III GSD

Case A was a 28 months old male child with an enlarged liver. Biochemical investigations of liver and muscle biopsies (Professor Hers et al. Louvain) showed high glycogen content in liver and moderately high in muscle. The polyarchozoa stored in both tissues had the structure of limit dextrin. Complete lack of amylol 1,6 glucosidase activity was demonstrated. Studies of glycogen content and amylol 1,6 glucosidase activity of erythrocytes gave concordant results.

Case B<sub>1</sub> was a 25 years old male with a history of dyspnoea and enlarged liver in childhood. He is now completely healthy with no liver enlargement.

Case B<sub>2</sub> a 9 months old male infant had a history of neonatal prolonged jaundice. Later on the liver was enlarged and he had attacks of convulsions responding to frequent feedings.

Case C<sub>1</sub> was a 7 years old male child. This case has been published previously (7). Biochemical investigations of liver biopsy (Professor Hers et al. Louvain) showed high glycogen content and complete lack of amylol 1,6 glucosidase activity.

Case C<sub>2</sub> a 2 years old brother of case C<sub>1</sub> had attacks of general convulsions during his first year of life. He still has an enlarged liver.

Case D<sub>1</sub> was a 21 months old male child with an enlarged liver.

In cases B<sub>1</sub>, B<sub>2</sub>, C<sub>1</sub> and D<sub>1</sub> the type diagnosis was made by erythrocyte studies (Fig. 1 and Table 4).

## METHODS

About 10 ml venous blood specimens were collected on heparin (preserved with chloroacetal 0.15%) and stored at 0°C. Within 2 days an erythrocyte suspension was obtained from the blood specimens as described by Van Hoof (15). The precipitate of washed erythrocytes was resuspended in 1 volume of isotonic NaCl and kept in a deep freezer until further processed. After thawing the hemolysis was centrifuged for 15 min at 10 000 × g. The supernatant was poured into an ice cooled test tube and stored for a few seconds followed by removal of 4.7 aliquots of 0.2 ml for the determination of amylol 1,6 glucosidase activity. Two aliquots of 25 µl were used for the determination of hemoglobin as cyanmethemoglobin (11). Aliquots of 2.0 ml were frozen for the determination of glycogen content.

Amylol 1,6 glucosidase activity was assayed by the incorporation of (U-C) glucose into glycogen (5). 0.2 ml of hemolysate together with 0.2 ml of a 70 (w/v) glycogen solution and 0.05 ml (U-C) glucose (15 µCi specific activity 1 µCi/µmole) was incubated at various lengths of time up to 4 hours. At the end of the incubation 0.5 ml of 15 M trichloroacetic acid and 2 ml of distilled water were added under continuous agitation followed by centrifugation. The glycogen was alcoholprecipitated 3 times before digestion with 2 ml of 20% KOH and a further 3 times afterwards. The final precipitate was dissolved in 0.7 ml water of which 0.5 ml was used for the radioactivity determined by liquid scintillation counting. The results were expressed as units per gram of hemoglobin, one unit being the amount of enzyme incorporating 0.1% of the counts added as glucose in 1 hour (15).

The glycogen was extracted from hemolysate with cold trichloroacetic acid as described by Van Hoof (15). After dialysis and reduction of the volume treatment with sakamycin dihydrochloride was performed and was followed by the glucose oxidase reaction. The glycogen was then calculated.

## RESULTS

Pedigrees and data concerning the activity of amylol 1,6-glucosidase activity of the members of the four families are shown in Fig. 1. Table 2 summarizes the results of the determinations of amylol 1,6 glucosidase activity of erythrocytes in 5 patients, the different groups of relatives and the 15 healthy controls. A total of 22 determinations on different samples from the 15 healthy controls were performed. No

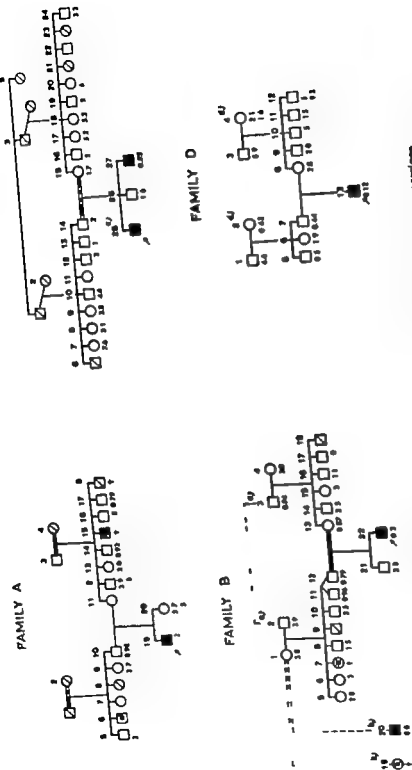


Fig. 1. Pedigrees of 4 families with glycogen storage disease type III.

- Male  
○ Female  
◻ Consanguineous marriage  
a) Persons B<sub>2</sub> and the mother of person B<sub>3</sub> are second and third cousins through different lines  
b) Persons B<sub>10</sub> and B<sub>10</sub> are third cousins to person B<sub>12</sub> and to each other. These three persons are also more distantly related to each other in a complicated manner  
c) Specimen from liver examined by professor Hers & at Louvain  
d) Distantly related to person A<sub>11</sub>

- Male  
Female  
◻ Studied relatives  
◻ Glucose activity (units/g hemoglobin) of erythrocytes  
◻ Patient with glycogen storage disease type III  
◻ Not studied relatives  
◻ Probable glycogenesis  
◻ Stillborn  
◻ Died in infancy



Table 3 *Amylo 16 glucosidase activity of red blood cells before and after a meal in three healthy adults*

Case	After overnight fasting (units/g Hb)	Two hours after a meal (units/g Hb)
GW	5.5	5.7
AT	3.1	3.1
PW	2.5	2.6

difference before and after a meal was found in 3 of the healthy subjects (Table 3). Five determinations were performed in the control subject with the lowest initial value (1.7 units/g Hb) all the subsequent 4 values being higher than the initial. The mean of the first determination in each of the 15 controls was 4.10 units/g Hb (Table 1). The distribution of the normal material showed two populations as there was a smaller population of high values. A similar observation was made by Van Hoof (15). The mean—2 SD can therefore not be used in calculating the lower border of normal values. However, none of the 22 values in the 15 healthy controls were below 1.7 units/g Hb.

The mean values of the 5 patients and of their 8 parents were 0.098 and 1.23 units/g Hb respectively. There was no overlapping between the two groups. Six of the 8 parents had amylo-16 glucosidase activity of erythrocytes below 1.3 units/g Hb (Fig. 1).

The activity of amylo-16 glucosidase of erythrocytes from 20 of a total of 57 relatives of the patients was below 1.7 units/g Hb (Fig. 1). Nine of the 20 values were below 1 unit/g Hb.

Table 4 *Glycogen concentration of erythrocytes from patients with GSD type III*

Patients*	Glycogen in erythrocytes $\mu$ g/g Hb
A <sub>15</sub>	311
B <sub>9</sub>	365
B <sub>20</sub>	1040
C <sub>17</sub>	224
D <sub>18</sub>	264

\* For identification of the patients see Fig. 1.

Consanguinity occurred in 3 of the families (A, B and C). Family D was distantly related to family A through two different lines (Fig. 1). Table 4 shows the glycogen content of erythrocytes in 5 of the patients with GSD type III. Studies of the relatives of the patients and of the healthy controls failed to show reproducible values of any magnitude.

## DISCUSSION

The method of determination of amylo-16 glucosidase activity appears to be reproducible. Samples taken from the same subject before and after a meal showed almost the same values (Table 3). Short lasting changes in the amylo-16 glucosidase activity of the erythrocytes seem therefore unlikely. Larger variations were found when some months elapsed between the different times of sampling from the same person. The levels were, however, about the same.

Our data on amylo-16 glucosidase activity of erythrocytes from patients with type III GSD and their parents corresponded well with the mean values (0.102 and 1.01 units/g Hb respectively) reported by Van Hoof (16). Our normal material had a higher mean and a larger range than that reported by Van Hoof in spite of the fact that the same technique was used in the two investigations. Whether this is due to hereditary or environmental differences is difficult to say. It is more likely due to difference in the branching of the glycogen used for the incubation or in some other factors included in the procedure.

The differences between the means of enzyme activity of the patients versus healthy controls and of parents versus healthy controls were relatively larger in this study than reported by Van Hoof. The mean activity of 8 parents was only 30% of that of healthy controls compared to 55% observed by Van Hoof.

From several studies (2, 14, 15, 18) including the present results it appears that probable heterozygotes may be detected by studying the amylo-16-glucosidase activity in the blood.

corpuscles from relatives of patients with GSD type III. In our pooled data on 57 relatives of the patients more than one third had amylo-1-6-glucosidase activity of erythrocytes below the lowest activity observed in the healthy controls. Our material is the largest number of relatives in a single investigation and included siblings, parents, siblings of the parents and paternal and maternal grandparents. Huijng *et al.* (6) were unable to detect heterozygotes by studying the overall activity of the leucocyte debranching system in 31 relatives of patients with GSD type III. All the relatives showed values within the normal range. The discrepancy between their results and those of other groups (2, 14, 18, 19) may well be caused by the use of different techniques in the leucocyte studies. Huijng *et al.* (6) measured the glucose production from phosphorylase limit dextrin while the other authors used incorporation of glucose into glycogen by the reverse reaction of amylo-1-6-glucosidase. This latter technique was also used in the erythrocyte studies performed by Van Hoof and in the present investigation. According to Huijng *et al.* both amylo-1-6-glucosidase and maltotriose-1-6-glucosyltransferase are required in their test system while the incorporation of glucose into glycogen measures only the amylo-1-6-glucosidase reaction. The authors suggested that the overall reaction would not be affected in their test system as long as the transferase activity is still limiting.

Van Hoof (15) demonstrated that the glycogen content of erythrocytes of heterozygotes was intermediary between normal and pathological situations although the results varied greatly from one examination to another in the individual cases. High glycogen content of erythrocytes was also found in the patients with type III GSD in this investigation. In our hands however the determination of glycogen content was not suitable for genetic studies.

The biochemical changes in relatives of patients with GSD type III confirm the clinical assumption that most cases of this particular type of GSD are inherited by an autosomal

recessive trait. In 1966 Brandt & De Luca (1) reported GSD type III in a mother and her 3 children suggesting a dominant type of inheritance. Since the parents were first cousins however a recessive type of inheritance was also considered.

The existence of subgroups of GSD type III in which the defect is incomplete has been demonstrated by several authors (2, 4, 8, 16). In 1967 Van Hoof & Hers (16) classified GSD type III into 4 subgroups based on biochemical analyses of liver and muscle biopsies. Brandt & De Luca (1) suggested that leucocyte studies must also be considered in this subgrouping. Although the data concerning the subgroups are still confusing the different degrees and localisations of the enzyme defect in patients with GSD type III make it difficult to exclude the possibility of a dominant type of inheritance in some of the cases.

A high incidence of consanguinity has been reported in families with cases of GSD type III indicating a recessive type of inheritance (1, 10, 12). Consanguinity occurred in 3 of the 4 families with GSD type III examined by us and there was a familial relationship between 2 of them. This probably explains the occurrence of 6 definite and 2 probable cases of GSD type III in a relatively small population (about 250 000 live births). In a comprehensive study Öckerman (20) was only able to trace a total of 13 definite or highly probable cases of GSD in Sweden. This implied an incidence of 1/246 000 live births. Only one of his cases was definite and one tentative type III.

This particular type of inborn error of metabolism seems to offer special advantages for genetical investigations. It should be possible to detect families with probable heterozygote carriers by performing screening studies in isolated areas with known cases of GSD type III. Enzymatic studies may also be of value in genetic counselling. The overlapping between normal and heterozygote values of amylo-1-6-glucosidase activity must, however be borne in mind.

Table 3 *Amylo-1 6 glucosidase activity of red blood cells before and after a meal in three healthy adults*

Case	After overnight fasting (units/g Hb)	Two hours after a meal (units/g Hb)
GW	5.5	5.7
KT	3.1	3.1
PW	2.5	2.6

difference before and after a meal was found in 3 of the healthy subjects (Table 3). Five determinations were performed in the control subject with the lowest initial value (1.7 units/g Hb), all the subsequent 4 values being higher than the initial. The mean of the first determination in each of the 15 controls was 4.10 units/g Hb (Table 1). The distribution of the normal material showed two populations as there was a smaller population of high values. A similar observation was made by Van Hoof (15). The mean—2 SD can therefore not be used in calculating the lower border of normal values. However, none of the 22 values in the 15 healthy controls were below 1.7 units/g Hb.

The mean values of the 5 patients and of their 8 parents were 0.098 and 1.23 units/g Hb respectively. There was no overlapping between the two groups. Six of the 8 parents had amylo-1 6-glucosidase activity of erythrocytes below 1.3 units/g Hb (Fig. 1).

The activity of amylo-1 6 glucosidase of erythrocytes from 20 of a total of 57 relatives of the patients was below 1.7 units/g Hb (Fig. 1). Nine of the 20 values were below 1 unit/g Hb.

Table 4 *Glycogen concentration of erythrocytes from patients with GSD type III*

Patients*	Glycogen in erythrocytes $\mu\text{g/g Hb}$
A <sub>1</sub>	311
B <sub>20</sub>	365
B <sub>24</sub>	1040
C <sub>17</sub>	224
D <sub>13</sub>	264

\*For identification of the patients see Fig. 1.

Consanguinity occurred in 3 of the families (A, II and C). Family D was distantly related to family A through two different lines (Fig. 1). Table 4 shows the glycogen content of erythrocytes in 5 of the patients with GSD type III. Studies of the relatives of the patients and of the healthy controls failed to show reproducible values of any magnitude.

## DISCUSSION

The method of determination of amylo-1 6 glucosidase activity appears to be reproducible. Samples taken from the same subject before and after a meal showed almost the same values (Table 3). Short lasting changes in the amylo-1 6 glucosidase activity of the erythrocytes seem therefore unlikely. Larger variations were found when some months elapsed between the different times of sampling from the same person. The levels were however about the same.

Our data on amylo-1 6 glucosidase activity of erythrocytes from patients with type III GSD and their parents corresponded well with the mean values (0.102 and 1.01 units/g Hb respectively) reported by Van Hoof (16). Our normal material had a higher mean and a larger range than that reported by Van Hoof in spite of the fact that the same technique was used in the two investigations. Whether this is due to hereditary or environmental differences is difficult to say. It is more likely due to difference in the branching of the glycogen used for the incubation or in some other factors included in the procedure.

The differences between the means of enzyme activity of the patients versus healthy controls and of parents versus healthy controls were relatively larger in this study than reported by Van Hoof. The mean activity of 8 parents was only 30% of that of healthy controls compared to 55% observed by Van Hoof.

From several studies (2, 14, 15, 18) including the present results it appears that probable heterozygotes may be detected by studying the amylo-1 6 glucosidase activity in the blood.

with glycog = storage disease *Clin Chim Acta* 8 807 1963

15 Van Hoof P Amylo-1-6-glycosidase activity and glycogen content of the erythrocytes of normal subjects patients with glycogen storage disease and heterozygotes *Europ J Bioch* 2 271 1967

16 Van Hoof P & Hers H □ The subgroups of type III glycogenosis *Europ J Bioch* 2 265 1967

17 Walke P O Sedberry J B Jr & Harris R C Hepatic phosphorylase defect Studies on peripheral blood *Am J Dis Child* 111 278 1966

18 Williams C & Field J B Studies in glycogen storage disease III Limit dextranosis A genetic study *J Pediatr* 72 214 1968

19 Williams H E Hendeg, E M & Field J B

Leucocyte debranching enzyme in glycogen storage disease *J Clin Invest* 42 656 1963

20 Öckerman, P A Glycogen storage disease in Sweden. *Acta Paediat Scand Suppl* 160 1965

Submitted Dec. 22 1969

Accepted Febr 17 1970

(P H W) Dept of Paediatrics  
Haukeland sykehus  
Bergen  
Norway

*Key words* Glycogen storage disease type III genetic studies consanguinity amylo-1-6-glycosidase activity in erythrocytes

Our material demonstrates that in most cases it is possible to diagnose GSD type III without performing liver and muscle biopsies. Two of Van Hoof's (15) patients with this disorder however showed an amylo-1,6 glucosidase activity of erythrocytes within or above normal limits. If Van Hoof's and our materials are pooled together 27 of a total of 29 cases showed a homozygote level of amylo-1,6 glucosidase activity in erythrocytes with the incubation technique.

### ACKNOWLEDGMENT

We acknowledge with gratitude the financial support received from Nasjonalfondet og de dermed forbundne fond to this research project. We also wish to express our thanks to colleagues of other Norwegian Hospitals, who kindly provided information about cases of glycogen storage disease and to professor H. G. Hers and Dr F. Van Hoof, Laboratoire de Chimie Physiologique, Université de Louvain, Belgium, for studies of biopsies performed in 2 of our cases. The technical assistance of Halvard J. Halvorsen, Department of Biochemistry, on a few occasions of this work is also acknowledged.

### SUMMARY

In 20 cases of glycogen storage disease (GSD) from 14 families in Norway genetic studies were performed in 4 families with GSD type III. Consanguinity occurred in 3 of the 4 families and familial relationship existed between 2 of them. Six cases in the 4 families had clinical findings compatible with a diagnosis of GSD. Homozygote values of amylo-1,6 glucosidase activity were found in erythrocytes from 5 studied cases. Family histories disclosed that 2 relatives of the patients died with probable GSD.

The mean amylo-1,6 glucosidase activity of erythrocytes from 8 parents of the patients was only 30% of that of healthy controls. Moreover 20 out of 57 relatives of the patients had low enzyme activity suggesting that they were heterozygotes. These relatives included siblings, parents, siblings of parents and grandparents.

The results are compatible with the assumption

that GSD type III is inherited by an autosomal recessive trait.

In most cases of GSD type III the diagnosis can easily be made by determining the amylo-1,6 glucosidase activity in erythrocytes from the patients. This type seems to offer special advantages for genetic studies.

### REFERENCES

- 1 Brandt I. K. & De Luca V. A. Type III glycogenosis. A family with an unusual tissue distribution of the enzyme lesion. *Amer J Med* 40: 779, 1966.
- 2 Chayoth R, Moses S. W. & Steinitz K. Debrancher enzyme activity in blood cells of families with type III glycogen storage disease. A method for diagnosis of heterozygotes. *Israel J Med Sci* 3: 422, 1967.
- 3 Cori G. T. Glycogen structure and enzyme deficiencies in glycogen storage disease. *Harvard Lectures Series* 48: p. 145, 1952-53.
- 4 Hers H. G. Amylo-1,6 glucosidase activity in tissues of children with glycogen storage disease. *Biochem J* 76: 69, 1960.
- 5 Hers H. G., Verheij W. & Van Hoof F. The determination of amylo-1,6 glucosidase. *Europ J Biochem* 2: 257, 1967.
- 6 Huang F., Klein O., Boudry, H. J. & van Creveld S. The activity of the debranching-enzyme system in leucocytes. A genetic study of glycogen storage disease type III. *Acta genet (Basel)* 18: 128, 1968.
- 7 Høyer S. E. & Moe P. J. Glykogenose. *Tidsskr Norste Lægeforen* 87: 614, 1967.
- 8 Illingworth B. A. & Brown D. H. Glycogen storage disease (types III, IV and VI) in W. J. Whelan & M. P. Cameron (eds.) *Control of glycogen metabolism*. Ciba Foundation Symposium 13 & A. Churchill Ltd. London, 1964: p. 336.
- 9 Kluge T. Glycogen storage disease. A case of generalized glycogenosis type 2 (Pompe's disease). *Acta Path. Microbiol. Scand* 63: 340, 1965.
- 10 Levin M., Moses S. W., Chayoth R., Jagoda N. & Steinitz K. Glycogen storage disease in Israel. A clinical, biochemical and genetic study. *Israel J Med Sci* 3: 397, 1967.
- 11 Moe P. J. Iron requirements in infancy. Longitudinal studies of iron requirements during the first year of life. *Acta Paediatr. Scand. Suppl.* 150, 1963.
- 12 Sidbury J. B. Jr. The genetics of the glycogen storage diseases. In A. G. Steinberg & A. G. Bearm (eds.) *Progress in medical genetics*. Grune & Stratton Inc. New York, 1965: vol. IV: p. 32.
- 13 Skallerud A. Myopathy ved glykogenose. *Nord Med* 81: 379, 1969.
- 14 Steinitz K., Bodur H. & Arman T. Amylo-1,6 glucosidase activity in leucocytes from patients

Table 1. Poliovirus antibody status 3 years after vaccination with trivalent oral vaccine

Antibody titre ≥ 1/10	Group of children	
	I (oral vaccine only)	II (inactivated vaccine + oral vaccine)
Against all three types	12/23 <sup>a</sup> (19/23)	18/23 <sup>b</sup> (23/23)
Against type 1	25/23 <sup>a</sup> (24/23)	19/23 <sup>b</sup> (23/23)
Against type 2	31/31 (12/31)	29/30 (30/30)
Against type 3	16/31 (28/31)	26/30 (28/30)
No detectable antibody	1/28 (0/28)	0/23 (0/23)

<sup>a</sup> Smaller number of children with antibody. Denominator number of children investigated. <sup>b</sup> Not a technical error: five blood samples in group I given as group II were not tested against poliovirus 1.

(Numbers in brackets: results with sera from the same children 1 month following the vaccination in 1966.)

In the present study the most prominent finding is the lack of demonstrable antibody against poliovirus type 3 in 17 of the 33 children in group I as compared with only five negatives among the same children 1 month after the vaccination in 1966.

The lack of antibody may be due to an initial response with lower antibody titre against the type 3 component of the vaccine, or due to a more pronounced fall in antibody titre for type 3 than for types 1 and 2.

The geometric mean antibody titre for children in group I in 1966 was lower for type 3 than for types 1 and 2, i.e. 1/86, 1/137 and 1/40 against types 1, 2 and 3 respectively. In order to compare the decline in antibody titre children with the same titre in 1966 against one virus type were grouped together. Starting from equal titre values in 1966 more children seemed to have lost antibody against type 3 than against types 1 and 2. Accordingly there is an indication that both of the mechanisms suggested above may have been operating. The number of children investigated is small and the antibody titres were determined in 1966 and 1969 respectively and by somewhat different techniques. The

results therefore do not permit firm conclusions.

However the results certainly indicate that further investigations of the antibody status after oral vaccination is warranted and such investigations become more important in view of the fact that oral vaccine has been used extensively in Norway since the autumn of 1965.

## SUMMARY

Neutralizing antibodies against poliovirus were investigated in 63 10-year-old children 3 years after oral poliovirus vaccination. The antibody status was compared with the results obtained when the same children were examined 1 month after the oral vaccination.

A marked change had taken place in the antibody status against poliovirus type 3 among the 33 children who had received only oral vaccine. Seventeen of them lacked demonstrable antibody against type 3 after 3 years as compared with only four negatives 1 month after the vaccination. The antibody status for these children against poliovirus type 1 and 2 had not undergone any major change. Possible mechanisms for this difference are briefly discussed.

The 30 remaining children had received inactivated poliovirus vaccine before the oral vaccination and for these children no major changes were observed in the antibody status against the three poliovirus types.

## ACKNOWLEDGEMENT

The interested co-operation of Dr Roarung Øvrelid, School of Medical Officers of Oslo, and the school nurses is greatly appreciated.

## REFERENCES

1. Cesaro T. C., Jones V., Poland J. D. & Chan T. D. Y. Persistence of neutralizing antibodies four years after immunization with live attenuated vaccine. *Amer J Epidemiol* 90: 157, 1969.
2. Just M. & Ruloff G. Die Immunität gegen Poliovirus 6 bzw. 8 Jahre nach oraler Impfung. *Schweiz Med Wochschr* 94: 1710, 1964.

## POLIOVIRUS ANTIBODY STATUS THREE YEARS AFTER VACCINATION WITH TRIVALENT ORAL VACCINE

I ØRSTAVIK AND LIV B FLUGSRUD

*From the Virus Department A (Head O Lohelle) National Institute of Public Health  
Oslo Norway*

In 1966 the serologic response to oral poliovirus vaccination was investigated in a group of school children in Oslo (7). The antibody status of the same children 3 years after the vaccination is presented in this communication.

### MATERIAL AND METHODS

In the previous study blood samples were collected from 80 children then aged 7 years before and 1 month after completed vaccination with three doses of trivalent oral poliovirus vaccine. According to the manufacturer (Glaxo Laboratories Ltd) one dose of vaccine contained 10 TCID<sub>50</sub> of type 1 poliovirus 10<sup>4</sup> TCID<sub>50</sub> of type 2 and 10 TCID<sub>50</sub> of type 3.

In February 1969 new blood samples were collected from 63 of the 80 children. Of these 63 children 33 had received only the oral vaccine (group I) whereas 30 children had also been given inactivated poliovirus vaccine in infancy (group II).

The collection of the blood samples on filter paper discs and the further treatment were performed as described previously (7). Two alterations were adopted for the neutralization tests: (a) Wild poliovirus strains were replaced by the attenuated Sabin strains. No difference in serum titre had been detected in preliminary experiments. (b) The serum virus mixture was incubated for 3 hours at 37°C and then overnight at 4°C before inoculation into monkey kidney tissue culture tubes. This technique is more sensitive than the incubation for 1 hour at 37°C used in the earlier assays.

The amount of blood absorbed by the filter discs is sufficient for only one examination by the neutralization test. The antibody status in 1969 is therefore compared with the results obtained after the vaccination in 1966.

### RESULTS

The results of the serological investigations are summarized in Table 1 where the two groups of children are considered separately. The corresponding results obtained in 1966 for the same children, are also presented.

A remarkable finding is the antibody status against poliovirus type 3 among the children in group I: more than half of them being without demonstrable antibodies (titre < 1:10) whereas most of these children have antibodies against types 1 and 2. Most of the children in group II have antibodies against all three virus types.

In group I one child is without demonstrable antibodies against all three virus types 3 years after vaccination.

In the same group one more child possesses antibody against poliovirus type 1 in 1969 as compared with the status 3 years previously, probably because of a poliovirus infection in the interval.

### COMMENTS

Several authors have reported a decline in antibody titre during the years following oral poliovirus vaccination. The antibody deficit has been most pronounced for poliovirus type 3 (1-6).

## LIVER FUNCTION STUDIES AND BILIARY TRACT INVESTIGATIONS IN MUCOVISCIDOSIS

JEAN FEIGELSON YVETTE PECAU and JACQUES SAUVEGRAIN

Paris, France

abnormalities of the liver and biliary tract have recently been reported in cystic fibrosis of pancreas (2 3 4 5 7 8 18 20 24 25 32 35). Liver cell atrophy fatty infiltration perportal fibrosis and proliferation of the bile ducts have been found. Small shrunken gallbladders have been described in about 23% cases studied (5 8) including small children and therefore may be congenital in origin. In a series of 30 children with cystic fibrosis we found that 11 of the patients manifested a loss of abdominal pain which were occasionally severe. The attacks might have retarded the development of pancreatic or intestinal lesions referred abdominal pain from biliary lesions or hepatic or gallbladder disease. Since 5% of such patients develop cirrhosis of the liver we studied liver function tests and made radiological investigations of the biliary tract in the hope of earlier detection of liver disease.

### MATERIAL AND METHODS

Thirty patients with cystic fibrosis (11 males and 19 females) aged 18 months to 18 years were studied. The diagnosis was established by clinical criteria chest X-ray findings stool analyses and sweat chloride determination (Table 1). Two children (patients 9 and 30) had overt clinical symptoms of cirrhosis of the liver.

The following tests were routinely performed serum glutamate-oxaloacetate and glutamate pyruvate transaminase (SGOT & SGPT) (13) thymol

turbidity MacLagan test (19) prothrombin test (22) and alkaline phosphatase levels (14). Elimination rate of bromosulphalein (BSP) was determined by estimation of the clearance of the dye after collection of serial blood specimens between 0 and 15 min after injection (10 17 21).

Radiological investigations of the biliary tract included oral cholecystography and in those cases in which satisfactory opacification of the bile ducts was not obtained intravenous cholangiography with radionuclides.

### RESULTS

The clinical radiological and biochemical findings are presented in Table 1. *Liver function tests*. Thymol turbidity and transaminases (SGOT & SGPT) were normal in all cases except two (nos 22 and 29) who had elevated levels during an attack of infectious hepatitis. Serial determinations of prothrombin were usually normal between 70 and 100% but in some instances fell to 40. In patient 20 aged 4 who had cirrhosis and had been operated on for neonatal ileus the prothrombin level fell as low as 28% 2 months before his death. Clearances of BSP were significantly abnormal in the two overtly cirrhotic patients. BSP half life was 17 min in patient 20 and 15 min in patient 30. BSP half life was slightly more than the normal level (5.5 min) in 5 patients nos 1 10 12 21 26. Seven children (patients 8 12 19 20 24 27 30) had alkaline phosphatase levels over 13 Bodansky



- 3 Melnick J L, Burkhardt M, Taber L H & Erckmann P N. Developing gap in immunity to poliomyelitis in an urban area. *JAMA* 209: 1181, 1969.
- 4 du Pan R M, Bayaud Ch & Koehli B. Bilan de l'immunité de la population scolaire de Genève après quatre ans de vaccination orale contre la poliomyélite. *11th Europ Sympos of Poliomyelitis* 1966. Masson & Cie Paris 1967 p 173.
- 5 Vonka V, Janda Z, Simon J, Adam E & Stárek M. A new type 3 attenuated poliovirus for possible use in oral poliovirus vaccine. *Progr Med Virol* 9: 204, 1967.
- 6 WHO Memorandum. Evidence on the safety and efficacy of live poliomyelitis vaccines currently in use with special reference to type 3 poliovirus. *Bull WHO* 40: 925, 1969.
- 7 Ørstavik I, Flugsrud Lav B & Løbelk O. Poliovirus antibody status and the response to inactivated oral poliovirus vaccine in Norwegian school children. *Acta Paediat Scand* 57: 405, 1968.

Submitted Jan 30 1970

Accepted Febr 26 1970

(I Ø) Virusavdeling A  
Statens Institutt for Folkehelse  
Geitmyrsveien 75  
Oslo 1 Norway

**Key words:** Oral poliovirus vaccine, neutralizing antibodies, duration of immunity.

# LIVER FUNCTION STUDIES AND BILIARY TRACT INVESTIGATIONS IN MUCOVISCIDOSIS

JEAN FRIGELSON YVETTE PECAU and JACQUES SAUVEGRAIN

Paris France

abnormalities of the liver and biliary tract have frequently been reported in cystic fibrosis of the pancreas (2 3 4 5 7 8 18 20 24 25 30 32 35). Liver cell atrophy fatty infiltration periportal fibrosis and proliferation of the bile ducts have been found. Small shrunken gallbladders have been described in about 23% of cases studied (5 8) including small children (5) and therefore may be congenital in origin. In a series of 30 children with cystic fibrosis we found that 11 of the patients manifested attacks of abdominal pain which were occasionally severe. The attacks might have retarded the development of pancreatic or intestinal lesions referred abdominal pain from pulmonary lesions or hepatic or gallbladder disease. Since 9% of such patients develop cirrhosis of the liver we studied liver function tests and made radiological investigations of the biliary tract in the hope of earlier detection of liver disease.

## MATERIAL AND METHODS

Thirty patients with cystic fibrosis 10 males and 20 females aged 18 months to 18 years were studied. The diagnosis was established by clinical criteria chest X-ray findings stool analyses and sweat chloride determinations (Table 1). Two children (patients 10 and 30) had overt clinical symptoms of cirrhosis of the liver.

The following liver function tests were routinely performed serum glutamic-oxaloacetic and glutamic pyruvic transaminase (SGOT) (23) thymol

turbidity MacLagan test (19) prothrombin test (22) and alkaline phosphatase levels (14). Elimination rate of bromsulphthalein (BSP) was determined by estimation of the clearance of the dye after collection of serial blood specimens between 0 and 15 min after injections (10 17 21).

Radiological investigations of the biliary tract included oral cholecystography and in those cases in which satisfactory opacification of the bile ducts was not obtained intravenous cholangiography with radiopaque contrast.

## RESULTS

The clinical radiological and biochemical findings are presented in Table 1. Liver function tests Thymol turbidity and transaminases (SGOT & SGPT) were normal in all cases except two (nos 22 and 29) who had elevated levels during an attack of infectious hepatitis. Serial determinations of prothrombin were usually normal between 70 and 100 but in some instances fell to 40%. In patient 20 aged 4 who had cirrhosis and had been operated on for neonatal ileus the prothrombin level fell as low as 28% 2 months before his death. Clearances of BSP were significantly abnormal in the two overtly cirrhotic patients. BSP half life was 17 min in patient 20 and 15 min in patient 30. BSP half life was slightly more than the normal level (5.5 min) in 5 patients nos 1 10 12 21 26. Seven children (patients 8 12 19 20 24 27 30) had alkaline phosphatase levels over 13 Bodansky

Table 1 Comparison of clinical findings radiological and laboratory results

Case no	Sex	Sweat test Cl mEq/l	Age in y at X ray	Pulm & gen condition	Cyclic abdominal pain	Liver symptoms	RSP b/m/l min
<i>Normal gallbladder</i>							
1	♀	107	4	Moderate	+	—	64
2	♀	110	7	Severe	—	—	45
3	♀	97	2½	Moderately well	—	—	4
4	♀	113	12	Moderately well	—	—	4
5	♀	124	3	Good	—	—	4
6 (†)	♀	132	7	Severe	+	—	3
7	♀	125	9	Moderate	+	—	4
8	♀	150	9	Good	—	—	5
9	♂	102	13	Moderately well	+	—	35
10	♀	116	4	Good	+	—	6
11	♀	145	10	Good	+	—	35
12	♀	105	5	Moderately well	+	Hepatosplenomegaly	7
13	♂	86	3	Good	—	—	4
14	♀	101	3	Good	—	—	35
15	♂	126	12	Moderately well	—	—	mal m 2 bases
16	♀	95	1½	Good	—	—	—
17	♀	137	7	Moderately well	+	—	5
<i>Microgallbladder</i>							
18	♂	121	5	Moderately well	—	—	35
19	♀	87	2½	Good (Mec ileus)	—	—	—
20 (†)	♂	127	4	Severe (Mec ileus)	—	Cirrhosis (congenital)	17
21	♀	78	12	Moderate	—	—	6
22	♂	117	8	Moderate	+	—	5
23	♀	110	1½	Moderate	—	—	mal m 4 bases
24	♂	142	3	Good	—	—	4
25	♂	100	5	Good	—	—	5
26	♀	91	10	Moderate	—	—	7
<i>No visualization of the gallbladder</i>							
27	♂	110	2	Moderately well	—	—	mal m 2 bases
28	♀	125	9	Moderate	+	Hepatosplenomegaly	35
29	♂	139	18	Moderate	—	—	15
30	♂	104	7	Moderate	+	Cirrhosis (Oesoph varices)	—

units. The higher levels were found in the two cirrhotic patients, and in the patient operated on for intestinal atresia (no 19). A high alkaline phosphatase level was also found in patient 8 who had mild pulmonary symptoms, no hepatomegaly and was in good general condition. Radiological investigation. In 17 out of 26 patients the gallbladder appeared to be normal in size as evaluated by comparison with vertebral and rib outline (4, 13, 28). We con-

sidered as normal the gallbladders of 2 patients (8 and 10) which seemed slightly enlarged with slow emptying but presenting a normal duct on the final film. In 9 cases gallbladder morphology was definitely abnormal. It was small and shrunken from 1 to 1.5 cm long and 0.5 cm wide (Figs 1, 2 and 3). The best descriptive term for this finding was felt to be 'microgallbladder'. Dye concentration ability, homogeneity of the stain and contrac-

alkaline phosphatase		Prothrombin lower level in per cent
level	Higher level	
9		80
8.5		55
13		90
11.5		90
1		100
		100
30		45
19		100
7		80
11		80
7		85
18		75
13		80
10.5		100
9		60
9.5		90
		100
9.5		45
19.5		40
21		28
3		72
10		85
		80
16		65
10.5		90
12		85
17		72
7.5		90
		76
47		90

tion after a fatty meal were normal despite their size and morphology. This aspect was highly characteristic and specific in the children. We did not observe any intermediate state of doubtful morphology. The differentiation between a normal gallbladder and a microgallbladder could definitely be made in all cases. Irregularities of the microgallbladder outlines were present in 3 cases and could have been due to the presence of submucosal cysts similar



Fig. 2 Microgallbladder patient 20 18 months old

to those described by Esterly & Oppenheimer (8) (Fig. 2). Microgallbladders were demonstrable in both the patients with a history of meconium ileus. In 4 cases cholecystograms were negative (patients 27, 28, 29, 30) but in two of them the main biliary tract was opacified by contrast medium and appeared normal.

## DISCUSSION

No direct relationship was found between abdominal pain and radiological abnormalities of the biliary tract. Only 3 patients with radiological abnormalities had cyclic abdominal pain. One had a microgallbladder with no cyst and two had no visualization of the biliary tract. Eight children who suffered from abdominal pain had a normal gallbladder. Six children with microgallbladders including those with submucosal cysts did not have any abdominal cyclic pain.

There was a good correlation between BSP half life and the clinical hepatic stage. BSP test confirmed the diagnosis of cirrhosis in 2 patients. In 5 children BSP half life was between 6 and 7 min. This might suggest the first stage of an evolution toward cirrhosis and

Table 1 Comparison of clinical findings radiological and laboratory results

Case no	Sex	Sweat test Cl mEq/l	Age in y at X ray	Pulm & gen condition	Cyclic abdominal pain	Liver symptoms	BSP half hr
<i>Normal gallbladder</i>							
1	♀	107	4	Moderate	+	—	6.5
2	♀	110	7	Severe	—	—	4.5
3	♀	97	2½	Moderately well	—	—	4
4	♀	113	12	Moderately well	—	—	4
5	♀	124	5	Good	—	—	4
6 (†)	♀	132	7	Severe	+	—	3
7	♀	125	9	Moderate	+	—	4
8	♂	150	9	Good	—	—	5
9	♂	102	13	Moderately well	+	—	3.5
10	♀	116	4	Good	+	—	6
11	♀	145	10	Good	+	—	3.5
12	♀	105	5	Moderately well	+	Hepatosplenomegaly	7
13	♂	86	3	Good	—	—	4
14	♀	101	3	Good	—	—	3.5
15	♂	126	12	Moderately well	—	—	mal 2 b
16	♀	95	1½	Good	—	—	5
17	♀	137	7	Moderately well	+	—	5
<i>Microgallbladder</i>							
18	♂	121	5	Moderately well	—	—	3.5
19	♀	87	2½	Good (Mec ileus)	—	—	mal 2 b
20 (†)	♂	127	4	Severe (Mec ileus)	—	Cirrhosis (congenital)	17
21	♀	78	12	Moderate	—	—	6
22	♂	117	8	Moderate	+	—	5
23	♀	110	1½	Moderate	—	—	mal 2 b
24	♀	142	3	Good	—	—	4
25	♂	100	5	Good	—	—	5
26	♀	91	10	Moderate	—	—	7
<i>No visualization of the gallbladder</i>							
27	♂	110	2	Moderately well	—	—	mal 2 b
28	♀	125	9	Moderate	+	Hepatosplenomegaly	3.5
29	♂	139	18	Moderate	—	—	15
30	♂	104	7	Moderate	+	Cirrhosis (Oesoph varices)	mal 2 b

units. The higher levels were found in the two cirrhotic patients and in the patient operated on for intestinal atresia (no 19). A high alkaline phosphatase level was also found in patient 8 who had mild pulmonary symptoms, no hepatomegaly and was in good general condition. *Radiological investigation.* In 17 out of 26 patients the gallbladder appeared to be normal in size as evaluated by comparison with vertebral and rib outline (4, 13, 28). We con-

sidered as normal the gallbladders of 2 patients (8 and 10) which seemed slightly enlarged with slow emptying, but presenting normal duct on the final film. In 9 cases gallbladder morphology was definitely abnormal. It was small and shrunken from 1 to 1.5 cm long and 0.5 cm wide (Figs 1, 2 and 3). The best descriptive term for this finding was felt to be 'microgallbladder'. Dye concentration ability, homogeneity of the stain and contrast

function. The multiple factors which control alkaline phosphatase make difficult the interpretation of their increase. We have occasionally found low prothrombin levels. This supervision is important from a practical point of view as it can be corrected by vitamin K<sub>1</sub> administration.

There has been correlation between radiological abnormalities of the biliary tract and liver function tests in only 3 patients: patient 20 had meconium ileus, microgallbladder, cirrhosis and a BSP half life of 17 min; patient 30 had a cirrhosis, no visualization of the gallbladder and a BSP half life of 15 min; in patient 19 who was operated on for a neonatal ileus and had a microgallbladder the BSP could not be performed but the alkaline phosphatase level was high.

There was no relationship between gallbladder abnormalities demonstrated by radiological means and the patients' age. Normal and abnormal gallbladders were found at all ages. In patient 7 we performed a second cholecystogram 2 years after the first examination because of the development of recurrent and severe abdominal pain. The second cholecystogram was normal as had been the previous one. Nonetheless it holds true that there can still be a whole range of degrees of impairment of the gallbladder depending on the amount and the viscosity of the secreted mucus. Under certain conditions the output of a stony mucus may result in an occluded gallbladder at an advanced stage of the disease. We did not observe this alteration but we did note that in two of our patients with negative cholecystograms the contrast medium opacified the main biliary tree (28), a fact in favour of an obstruction of the accessory biliary tract. The lack of opacification of the gallbladder might also be a consequence of the alteration of the wall (16).

The microgallbladders observed were not radiologically occluded. They were functioning properly and capable of concentrating contrast media. Histological studies (5-8) have shown their mucosa free of inflammatory changes and

a high frequency of multilocular cysts in their submucosa. Those seen on X-ray pictures display what seems to be an overdevelopment of the same phenomenon. We therefore have to concur with Esterly & Oppenheimer (8) who considered the gallbladder to be either intact or affected in utero with no intermediate stage. The microgallbladder does not seem to be a consequence but rather an occasional primary congenital feature of cystic fibrosis. It can be supposed that this involvement of the gallbladder is a variant of the occasional changes of other organs with a common endodermal origin such as liver, intestine and pancreas.

The gallbladder is not by itself an essential organ. Cholecystograms are none the less worthy of interest in the study of pathophysiology of cystic fibrosis. The existence of microgallbladders is another witness of congenital lesions in this disease. Survival of patients with mucoviscidosis depends essentially on the state of their lungs. Hepatic supervision is however important: liver function tests permit us to confirm or to complete clinical impressions and facilitate in some cases the correction of certain deficiencies.

## SUMMARY

Liver function tests and radiological investigations of the biliary tract were performed in 30 patients with cystic fibrosis of the pancreas.

Bromsulphalein excretion tests (BSP half life) were performed in 27 children. They were normal in 20 children. 5 patients had moderate changes and 2 patients with cirrhosis had definitely pathological tests. Alkaline phosphatase levels were often increased. Serial determination of the prothrombin level was usually normal but fell in some instances to 40% of the normal and even as low as 28% in a cirrhotic patient.

Radiological investigation of the biliary tract showed marked changes in 13 patients. Four patients had no visualization of the gallbladder. In 9 cases cholecystograms showed a highly



Fig 2 Microcystic bladder with possible multilocular cysts patient 10 years (patient 26)

requires special supervision. In patient 6 BSP was performed 1 month before she died and autopsy did not show any hepatic lesion. There was a correlation between high alkaline phosphatase level and BSP half life increases in 3

patients: the two cirrhotic ones and the patient with a BSP half life of 7 min who had hepatosplenomegaly. However one child with a constant high alkaline phosphatase level was in good general condition and had normal hepat



Fig 3 Microcystic bladder (nodular form) boy 5 years of age (patient 25)

function. The multiple factors which control alkaline phosphatase make difficult the interpretation of their increase. We have occasionally found low prothrombin levels. This supervision is important from a practical point of view as it can be corrected by vitamin K1 administration.

There has been correlation between radiological abnormalities of the biliary tract and liver function tests in only 3 patients: patient 20 had meconium ileus, microgallbladder, cirrhosis and a BSP half life of 17 min; patient 30 had a cirrhosis, no visualization of the gallbladder and a BSP half life of 15 min; in patient 19 who was operated on for a neonatal ileus and had a microgallbladder, the BSP could not be performed but the alkaline phosphatase level was high.

There was no relationship between gallbladder abnormalities demonstrated by radiological means and the patients' age. Normal and abnormal gallbladders were found at all ages. In patient 7 we performed a second cholecystogram 2 years after the first examination because of the development of recurrent and severe abdominal pain. The second cholecystogram was normal as had been the previous one. Nonetheless it holds true that there can still be a whole range of degrees of impairment of the gallbladder depending on the amount and the viscosity of the secreted mucus. Under certain conditions the output of a stringy mucus may result in an occluded gallbladder at an advanced stage of the disease. We did not observe this alteration but we did note that in two of our patients with negative cholecystograms the contrast medium opacified the main biliary tree (28), a fact in favour of an obstruction of the accessory biliary tract. The lack of opacification of the gallbladder may also be a consequence of the alteration of the wall (16).

The microgallbladders observed were not radiologically occluded. They were functioning properly and capable of concentrating contrast media. Histological studies (5, 8) have shown the mucosa free of inflammatory changes and

a high frequency of multilocular cysts in their submucosa. Those seen on X-ray pictures display what seems to be an overdevelopment of the same phenomenon. We therefore have to concur with Esterly & Oppenheimer (8) who considered the gallbladder to be either intact or affected *in utero* with no intermediate stage. The microgallbladder does not seem to be a consequence but rather an occasional primary congenital feature of cystic fibrosis. It can be supposed that this involvement of the gallbladder is a variant of the occasional changes of other organs with a common endodermal origin such as liver, intestine and pancreas.

The gallbladder is not by itself an essential organ. Cholecystograms are none the less worthy of interest in the study of pathophysiology of cystic fibrosis. The existence of microgallbladders is another witness of congenital lesions in this disease. Survival of patients with mucoviscidosis depends essentially on the state of their lungs. Hepatic supervision is however important. Liver function tests permit us to confirm or to complete clinical impressions and facilitate in some cases the correction of certain deficiencies.

## SUMMARY

Liver function tests and radiological investigations of the biliary tract were performed in 30 patients with cystic fibrosis of the pancreas.

Bromsulphthalein excretion tests (BSP half life) were performed in 27 children. They were normal in 20 children. 11 patients had moderate changes and 2 patients with cirrhosis had definitely pathological tests. Alkaline phosphatase levels were often increased. Serial determination of the prothrombin level was usually normal but fell in some instances to 40% of the normal and even as low as 28% in a cirrhotic patient.

Radiological investigation of the biliary tract showed marked changes in 13 patients. Four patients had no visualization of the gallbladder. In 9 cases cholecystograms showed a highly





Fig 2 Microgallbladder with possible multilocular cysts girl of 10 years (patient 26)

requires special supervision. In patient 6 BSP was performed 1 month before she died and autopsy did not show any hepatic lesion. There was a correlation between high alkaline phosphatase level and BSP half life increases in 3

patients: the two cirrhotic ones and the patient with a BSP half life of 7 min who had hepatosplenomegaly. However, one child with a constant high alkaline phosphatase level was in good general condition and had normal hepatobiliary



Fig 3 Microgallbladder (another form) boy 5 years of age (patient 25)

function. The multiple factors which control alkaline phosphatase make difficult the interpretation of their increase. We have occasionally found low prothrombin levels. This superoinformation is important from a practical point of view as it can be corrected by vitamin K<sub>1</sub> administration.

There has been correlation between radiological abnormalities of the biliary tract and liver function tests in only 3 patients. Patient 1 had meconium ileus, microgallbladder, fibrosis and a BSP half life of 17 min. Patient 2 had a cirrhosis, no visualization of the gallbladder and a BSP half life of 15 min. In patient 19 who was operated on for a neonatal ileus and had a microgallbladder the BSP could not be performed but the alkaline phosphatase level was high.

There was no relationship between gallbladder abnormalities demonstrated by radiological means and the patients' age. Normal and abnormal gallbladders were found at all ages. In patient 7 we performed a second cholecystogram 2 years after the first examination because of the development of recurrent severe abdominal pain. The second cholecystogram was normal as had been the previous one. Nonetheless it holds true that there can still be a whole range of degrees of impairment of the gallbladder depending on the amount and the viscosity of the secreted mucus. Under certain conditions the output of a stringy mucus may result in an occluded gallbladder at an advanced stage of the disease. We did not observe this alteration but we did note that in two of our patients with negative cholecystograms the contrast medium opacified the main biliary tree (28) a fact in favour of an obstruction of the accessory biliary tract. The lack of opacification of the gallbladder might also be a consequence of the alteration of the wall (16).

The microgallbladders observed were not radiologically occluded. They were functioning properly and capable of concentrating contrast media. Histological studies (5, 11) have shown their mucosa free of inflammatory changes and

a high frequency of multilocular cysts in their submucosa. Those seen on X ray pictures display what seems to be an overdevelopment of the same phenomenon. We therefore have to concur with Eaterly & Oppenheimer (8) who considered the gallbladder to be either intact or affected in utero with no intermediate stage. The microgallbladder does not seem to be a consequence but rather an occasional primary congenital feature of cystic fibrosis. It can be supposed that this involvement of the gallbladder is a variant of the occasional changes of other organs with a common endodermal origin such as liver, intestine and pancreas.

The gallbladder is not by itself an essential organ. Cholecystograms are none the less worthy of interest in the study of pathophysiology of cystic fibrosis. The existence of microgallbladders is another witness of congenital lesions in this disease. Survival of patients with mucoviscidosis depends essentially on the state of their lungs. Hepatic supervision is however important. Liver function tests permit us to confirm or to complete clinical impressions and facilitate in some cases the correction of certain deficiencies.

## SUMMARY

Liver function tests and radiological investigations of the biliary tract were performed in 30 patients with cystic fibrosis of the pancreas.

Bromsulphthalein excretion tests (BSP half life) were performed in 27 children. They were normal in 20 children. 5 patients had moderate changes and 2 patients with cirrhosis had definitely pathological tests. Alkaline phosphatase levels were often increased. Serial determination of the prothrombin level was usually normal but fell in some instances to 40% of the normal and even as low as 28% in a cirrhotic patient.

Radiological investigation of the biliary tract showed marked changes in 13 patients. Four patients had no visualization of the gallbladder. In 9 cases cholecystograms showed a highly

characteristic finding a small shrunken microgallbladder with occasionally irregular contours presumably due to submucosal cysts

## REFERENCES

- Alagille D Le complexe prothrombique dans l'exploration fonctionnelle de la cellule hépatique *Rev Int Hepat* 4 1 1954
- Alagille D & Le Tan Vinh Les localisations hépatiques de la mucoviscidose *Gastro Enter* 4 435 1961
- Andersen D Cystic fibrosis of the pancreas and its relation to celiac disease clinical and pathological study *Amer J Dis Child* 56 344 1938
- Antonelli M Durio A & Marino P La colecistografia orale ed endovenosa in 17 bambini affetti da fibrosi caeca *Atti del XVIII Congresso Nazionale di Radiologia Medica e Medicina Nucleare* 4 293 Padova 1968
- Bodian M *Fibrocystic disease of the pancreas* Heinemann London 1952
- Brunnhage F & Enell H Mucoviscidos *Swensk Lakartidn* 4 1335 1961
- Craig J Haddad M & Schwachman H The pathological changes in the liver in cystic fibrosis of the pancreas *Amer J Dis Child* 93 357 1957
- Esterly J & Oppenheimer E Observation in cystic fibrosis of the pancreas I the gallbladder *Bull John Hopk Hosp* 110 247 1962
- Farber S Pancreatic function and disease in early life V Pathological changes associated with pancreatic insufficiency in early life *Arch Path* 37 238 1944
- Favre R Benhamon J P & Loverio A Considerations générales sur la notion de cirrhose hépatique *Rev Int Hepat* 7 429 1958
- Gracey M Burke V & Anderson C M Treatment of abdominal pain in cystic fibrosis by oral administration of n-acetyl cysteine *Arch Dis Child* 44 404 1969
- Hennequet A Mucoviscidose *Encycl med chir* 6 4060 1967
- Jones M Sakai H & Rogerson A Intravenous cholangiography in children with cystic fibrosis of the pancreas *J Pediatr* 59 172 1958
- Klem H Read P A & Baboon A A rapid method for quantitative determination of serum alkaline phosphatases *Clin Chem* 6 269 1960
- Kollberg H Diagnostik och behandling av mucoviscidos *Lakartidningen* 29 2929 1969
- La ser E *Dynamic factors in roentgen diagnosis* Williams & Wilkins Baltimore 1967
- Lindquist B & Paulsen L Studies on the elimination rate of bromsulphthalein in infants and children *Acta Paediat Scand* 48 233 1959
- Le Tan Vinh Cirrhose et mucoviscidose *Presse Med* 72 1934 1964
- MacLagan N F Thymal turbidity test as an indicator of liver dysfunction *Brit J Exp Pathol* 25 234 1944
- Nezelof C Données anatomopathologiques de la maladie fibrokystique du pancréas *Le Pédiatre Par* 11 7 1965
- Polonovski C & Colm J Explorations biologiques en pédiatrie Expansion Scientifique Française Paris 1963 p 602
- Quick A J On relationship between complement and prothrombin *J Immun* 29 17 1935
- Reitman S & Frankel S Colorimetric method for the determination of serum transaminase activity *Amer J Clin Path* 28 1 1957
- Sanes M Miller D Brasos F & Gest O Fatty changes and cirrhosis of the liver in patients with pancreatic lithiasis *Arch Int Med* 85 980 1950
- di Sant Agnese P & Blanc W A distinctive type of biliary cirrhosis of the liver associated with cystic fibrosis of the pancreas *Pediatrics* 3 387 1956
- di Sant Agnese P & Vidaurte A Cystic fibrosis of the pancreas *J Amer med Ass* 177 135 1960
- di Sant Agnese P & Talamo R Pathogenesis and physiopathology of cystic fibrosis of the pancreas *New Eng J Med* 277 1287 1967
- Sauvegrain J & Feigelson J La cholestylographie dans la mucoviscidose *Ann Radiol* 13 XXX 1970
- Schwachman H Kulczycki L & Koo Tai Khyu Studies in cystic fibrosis A report on 65 patients over 17 years of age *Pediatrics* 5 684 1965
- Turk B & Antonelli M Mucoviscidos con atresia epatica masiva *Rivista di Clinica Ped* 78 487 1966
- Vignon G Pansu D & Chapuy P Les ovaries decalcifiés par malabsorption de l'acide *Le Concours Med* 22 5733 1969
- Webster R & Williams H Hepatic cirrhosis associated with fibrocystic disease of the pancreas clinical and pathological reports of 5 patients *Arch Dis Child* 28 343 1953
- Williams T Sherman N & Clainworthy H Pancreatitis as cause of abdominal pain *Pediatr* 100 6 1019 1967
- Winkler H & Zollinger H Congenital cystic fibrosis with bronchiectasis *Helv Paediat Acta* 13 1945
- Zuelzer W & Newton W The pathogenesis of fibrocystic disease of the pancreas *Pediatrics* 4 53 1949

Submitted Aug 27 1969

Accepted March 18 1970

(J F) 49 Boulevard Beaumais 49

Paris 17

France

Key words Mucoviscidosis gallbladder microgallbladder BSP half life alkaline phosphatase prothrombin cirrhosis

## FAMILIAL ATAXIC DIPLEGIA WITH DEFICIENT CELLULAR IMMUNITY

### *A New Clinical Entity*

U HÅBERG O HANSSON S LIDÉN and K. NILSSON

*From the Departments of Pediatrics and Dermatology and the Wallenberg Laboratory  
University of Uppsala Uppsala Sweden*

In 1966 a girl with vaccinia gangrenosa successfully treated with N-methylsulfamyl-thiosemicarbazone (Marboran® Burroughs Wellcome & Co) was reported (8). During recovery at the age of 15 months she was found to have an ataxic diplegia which had not been observed previously. At that time it was not known whether the neurological defect was 1) a sequela of the severe disease 2) a toxic side effect of Marboran® or 3) unrelated to the case and its treatment.

Later on a younger brother who had not been vaccinated against variola was found to have an identical neurological syndrome. Both siblings also showed evidence of deficient cellular immunity. At the age of 4 3/4 years the child died of generalized varicella during steroid treatment of a hemolytic anemia. The post mortem examination reported elsewhere (1) revealed pathological findings in the central nervous system thought neither to be due to the final acute disease nor to the vaccinia gangrenosa or its treatment.

The aim of this paper is to complete the previous reports (1-8) with a detailed neurological follow up of the two siblings and with immunological data on the younger boy.

### CASE HISTORIES

#### *Case 1*

III H a girl born January 6 1962. First child of healthy non-related parents. No relatives with known

cerebral palsy or immunological disease. Pre- and neonatal history uneventful. Birthweight 3000 g.

As early as at the age of 9 months during hospitalization for the abnormal reaction to the variola vaccination the girl was considered late in her psychomotor development. At that time she was not able to turn round from prone to supine to creep or to sit unsupported. Her fine motor skill was more advanced. She could grasp adequately and switch over to the other hand. In spite of a good general condition during her first months in the local county hospital she did not learn to sit unsupported until about 12 months of age and even then she swayed and sat very unstably. As a result of her severe vaccinia gangrenosa she lay listlessly in her bed during that period.

At neurological examination when 17 months of age she was unable to sit without support. She showed generalized muscular hypotonia truncal tremor and pronounced difficulties to maintain posture in upright position i.e. dysequilibrium. She also showed a slight intention tremor in her hands and head. In addition saccadic clonus had been observed on some occasions.

After recovering from her vaccinia she started to develop but very slowly. At the age of 21 months an ataxic diplegia with spasticity of the feet was very apparent. The right foot was more spastic than the left. Her intellectual capacity was considered to be at a 12-month level and her speech poor. No squint was observed.

At 29 months she had learnt to kneel unsupported to stand unsteadily with support and to walk with two-hand support. Her arms and hands were much better developed than her legs and feet which functioned at about the 10-11 month level.

The last neurological examination was made when the girl was 4 1/2 years of age. At that time her neurological syndrome had become even more clear cut. She could now walk broad based without support. A marked valga insufficiency of her feet marked the tendency to tip-toe gait. Ataxia with pronounced dysequilibrium dominated the picture but

she had persistent ankle clonus and extensor plantar response in addition

Two months later she acquired hemolytic anemia and was treated with cortisone but unfortunately contracted chicken pox at the same time and died of a malignant generalized varicella. For a detailed autopsy report, see Berg et al (1).

#### Case 2

J A H a boy born September 7 1964 the younger brother of case 1. No other siblings. After a normal pregnancy he was born 10 days before term in a normal delivery. Birthweight 3890 g. Neonatal period uneventful.

At the age of 3 months he learned to smile and to fix his gaze. Otherwise his motor development was soon found to be retarded. He had poor head control and was not able to turn around at the age of 12 months. One month later he was sent in for neuropaediatric examination. He was then at 13 months of age found to function at the low motor level of about 2-3 months. His neonatal reflexes had disappeared but he had not yet any protection reflexes. He showed pronounced generalized hypotonia. At the same time he had brisk reflexes of the legs with a suspected ankle clonus. In addition a proprioceptive body dysfunction was thought probable but not yet significant.

At follow up examination when 19 months old he functioned at a motor level of 4 months. At the age of 2 1/2 years he was able to creep 10 m unsupported with an unsteady and bent back and to roll over. When put in a standing position he was able to stand with two hand support the knees however were markedly hyperextended and the feet were in a position of maximal valgus insufficiency. His mental development was slightly retarded but he was able to produce short sentences. Neurologically the main finding was pronounced hypotonia and a dysequilibrium syndrome with truncal tremor but no significant intention tremor. In addition the distal spasticity of his legs was confirmed by bilateral ankle clonus and extensor plantar response. He had no squint and his fundi were normal.

At the age of 4 1/2 years he had developed further and was able to walk supported by one hand but his gait was dysrhythmic with irregular abnormal steps. Protection reflexes in the legs were completely absent. His fine motor skill was at about the 2 1/2 year level and far ahead of the rest of his motor function. He obviously had different perceptual defects. This was most obvious in regard to his corporeal (body image) and extracorporeal awareness. The neurological picture was in all essential respects unchanged at this follow up. Mentally he was considered to be low normal or slightly subnormal.

The boy's general condition was good. He was not particularly prone to infections and he had gone through ordinary chicken pox without complications in 1966. Apart from his neurological abnormalities nothing abnormal was found on general physical examination.

## NEUROPHYSIOLOGICAL AND METABOLIC SPECIAL EXAMINATIONS

EEG was recorded four times (October 1965 April 1966 October 1966 and March 1969). The first three curves were within normal variations. The fourth curve recorded at the age of 4 1/2 years, showed somewhat slow background activity with a frequency of 7 c/sec but no paroxysmal abnormalities.

EMG findings were normal.

Conduction velocity rate: Right peroneal nerve 52 left 54 m/sec (normal).

Vibration tests: Pronounced spastic responses were obtained from the legs and the flexor muscles of the arms.

Metabolic screening on 12 hour night urine: The findings in various tube and tape tests were normal as also was the pattern at 2 dimensional amino acid chromatography.

## IMMUNOLOGICAL INVESTIGATIONS

The ability of the patient to develop contact allergy was tested by means of sensitization attempts with 2,4-dinitrochlorobenzene (DNCB). Twelve control persons (3 children and 9 adults) were given one sensitizing application of a solution of 2% DNCB in acetone (w/v). Twenty five  $\mu$ l of this solution (=0.5 mg of DNCB) was applied evenly to an area of 3 cm<sup>2</sup> on the back. This area was occluded by an impermeable plastic sheet when the solvent had evaporated. This occlusive dressing was kept in place for 48 hours as a rule but had to be taken off after 24 hours in one of the children because of an intolerable burning sensation. Simultaneously with the application of the sensitizing DNCB solution, a test of the primary toxicity of DNCB was performed by means of occlusive patch tests (Al test<sup>®</sup> Imco Astra Agency Co. Stockholm Sweden). In this test the allergen is applied on a piece of filter paper with an area of 0.8 cm<sup>2</sup>. These patches were moistened with 10  $\mu$ l of DNCB in absolute ethanol 0.15% 0.10% and 0.05% (w/v) respectively. The patch tests were left in place for 48 hours. Seventy-two hours after applica-

tion of the tests the response to the different concentrations of DNCB was evaluated. If any erythema occurred on any of the patch test sites the concentration of DNCB causing it was not used in the final testing (this happened in two cases with the 0.15% solution). In this way it was possible to obtain true positive patch test reactions (homogeneous erythema with palpable induration) 12–25 days after the sensitization in 11 out of the 12 control persons. The person not responding to the sensitizing attempt was a woman 65 years old. Most probably the failure can be explained by her age as it has been shown that elderly people become sensitized against DNCB less readily than younger people (17).

In our patient the first sensitization attempt failed and was therefore repeated this time with the amount of allergen doubled. Nevertheless no contact sensitivity was induced.

Intracutaneous injection of an extract of *Candida albicans* resulted in a non significant delayed reaction. The patient had never been vaccinated against tuberculosis and therefore no tuberculin test was performed.

Lymph nodes from the groin were excised for histopathological examination. These lymph nodes had not been deliberately stimulated with any antigen. They contained a few germinal centres and also plasma cells. The number of lymphocytes seemed to be decreased all over the node, a fact making it difficult to make a firm statement with regard to the development of the thymus-dependent paracortical areas and giving an impression of reticuloendothelial hyperplasia.

The differential counts of white blood cells gave rather low values of the lymphocytes as a rule about 500/mm<sup>3</sup>. The minimum value recorded was 350 lymphocytes/mm<sup>3</sup> and this occurred during an episode with acute pneumonia and otitis bilaterally.

Lymphocytes from the peripheral blood of the patient were cultured in and without the presence of phytohemagglutinin (PHA). Lymphocytes were separated from the blood as described by Thierfelder (16). The cells

(10<sup>6</sup>/ml) were incubated at 37°C in Eagles MEM supplemented with 20% autologous serum. 0.12 ml of a PHA-M (Difco) stock solution was added to each test tube for 72 hours and the cultures were then fed with tritiated thymidine (<sup>3</sup>H Tdr 1 µCi per culture) for 20 hours. The cultures were finally harvested according to a method advised by Ling (10) and the incorporation of <sup>3</sup>H Tdr into DNA was measured in a liquid scintillation counter (Packard). Included in the test were also triplet cultures of the patient's unstimulated lymphocytes, PHA-M stimulated normal adult lymphocytes as positive controls and unstimulated normal adult lymphocytes as negative controls. These cultures were incubated, fed with tritiated thymidine, harvested and counted as is described above for the patient's PHA stimulated cultures. The number of disintegrations per minute (c.p.m.) in the unstimulated cultures of the patient (mean 40) did not differ significantly from that of the PHA stimulated cultures (mean 38). Mean c.p.m. for the negative control cultures was 43 and 2000 for the positive ones.

The capacity of the patient to produce circulating antibodies was assessed by means of determinations of immunoglobulins and of isohemagglutinins. When the patient was 13 months old the value of immunoglobulin G (IgG) was subnormal (300 mg/ml). At 4½ years all immunoglobulin values tested (IgA, IgE, IgG, IgM) were normal. The isohemagglutinins were found to be low when he was 1½ years old (weak agglutination of A<sub>1</sub> erythrocytes with undiluted serum). At 5 years isohemagglutinins occurred even in a dilution of the serum of 1/8. The direct Coombs test was negative.

## DISCUSSION

The neurological syndrome of the two siblings was one of non progressive ataxic diplegia, a truncal tremor dominating the clinical picture. In all essential respects the abnormalities were the same in the two cases although more pronounced in the boy. The neurological picture

corresponded to the so called dysequilibrium state (7) dominated by proprioceptive and perceptual defects and with no or only minor components of intention tremor and dysmetria.

The etiology of ataxic forms of cerebral palsy remains in most cases obscure. Prenatal factors are considered to have been at work in the majority according to Ingram (9) prenatal factors are the cause of ataxic diplegia in about 50%. In familial cases of cerebral palsy, Gustavsson *et al* (6) found in a recent series that ataxic forms were much more frequent than other syndromes. The mode of inheritance in ataxic diplegia was considered to be autosomally recessive, autosomally dominant or sexlinked recessive. Our two cases are believed to belong to the first category.

In addition to their ataxic diplegia our two siblings also showed evidence of deficient cellular immunity. To our knowledge a familial syndrome of this type has not been reported previously. There are similarities to ataxia telangiectasia (Louis Bar syndrome). However clinically the well known features of that syndrome are clearly different from those in our cases. Neither telangiectasia nor the typical cerebellar ataxia was present. In addition to qualitative differences the ataxic diplegia of our patients appeared to be non progressive with reservation for the limited number of years of follow up. The histopathological picture in our autopsy case also differed from that in ataxia telangiectasia. In our girl the cerebellum was of normal size at autopsy while in the Louis Bar syndrome it is atrophied (2, 14, 15). In our case there was dysplasia of nerve cells in the cortex of the cerebrum and basal ganglia and heterotopic nerve cells were found in the white matter. In the cerebellum there was a patchy loss of Purkinje cells probably dysplasia. Non specific degeneration was seen in dorsal root ganglia and in the lateral corticospinal tract (1). These changes in the central nervous system do not tally with those described in ataxia telangiectasia (14, 15).

The deficiency in cellular immunity in the girl was proven by her failure to respond to

intracutaneous injection of vaccinia antigen in spite of the severe vaccinia gangrenosa (8).

The boy was judged to have a defect in his cellular immunity from the failure of the attempt at contact sensitization with DNCB and from the lack of response to PHA. The negative reaction to the test with candida antigen is compatible with this assumption. The system producing humoral antibodies on the other hand seemed to be essentially normal as judged from the normal values of the various immunoglobulins. Earlier the boy showed subnormal IgG values but these were by no means comparable to the very low values seen in hypogammaglobulinemic states (5). According to the delineation of the lymphoid system presented by Cooper *et al* (3) and by Parrot *et al* (12) the germinal centers belong to the part of this system involved in antibody production. According to this view the occurrence of germinal centers in the lymph nodes of our patient thus fits with the normal immunoglobulin levels in his serum. The number of lymphocytes in the peripheral blood was low on several occasions of determination. No real lymphopenic episodes were noted, however and it seems as if the possible deficiency in the lymphocyte production would be only moderate.

The immunological status of the patient thus showed a combination of a defect in his cellular immunity and on some occasions subnormal lymphocyte content in the peripheral blood but a fair production of immunoglobulins. A similar picture is seen in many cases of Louis Bar ataxia telangiectasia (13). Also these patients show impaired development of contact sensitivity and some of them have only minor defects in their immunoglobulin production. Further their lymph nodes contain follicular structures and often show a reticulo-endothelial hyperplasia.

From a purely immunological point of view the DrGeorge syndrome (4) must also be kept in mind. This syndrome is characterized by hypoparathyroidism and congenital absence of the thymus, normal levels of immunoglobulins

and of peripheral lymphocytes but with a defect in immunological competence particularly in cellular immunity. No investigations regarding the possible absence of the thymus were performed in our second patient. However the elder sister with the same clinical picture did have a thymus, a fact which certainly lessens the similarity with the immunological status in the DeGeorge syndrome.

Pure alymphocytosis (11) is also accompanied by thymic dysplasia and an impaired cellular immunity combined with normal immunoglobulins. The number of peripheral lymphocytes varied in the patient with this disease reported by Nezzelof (11) and on some occasions it was on the same level as in our patient.

It is not possible to differentiate the immunological components of the disease shown by our patient from the three above mentioned syndromes by means of the investigations performed. The probability of a hereditary trait and the presence of neurological disturbances make a comparison with ataxia telangiectasia, the only hereditary neurological syndrome with immunological deficiency hitherto described most relevant. However the neurological features of our two cases clearly distinguish their disorder from the Louis Bar syndrome.

### SUMMARY

Two siblings, a girl and a boy with an ataxic diplegia and deficient cellular immunity are reported. On the basis of the familial occurrence of this syndrome a hereditary genesis seemed likely. The girl died of varicella during steroid treatment for hemolytic anemia. Neuropathological examination revealed multiple dysplastic changes supporting a prenatal cause of the neurological syndrome. This combined neurological and immunological disorder is thought to be a new clinical entity.

### ACKNOWLEDGEMENTS

The investigation was supported by grants from the Folke Bernadotte Foundation and the Swedish Medical Research Council (Grant No. D68 19X 108 05).

### ADDENDUM

After this paper was prepared Case 2 died at the age of 5 1/2 years. Three months before his death an acute hemiplegia of the right arm and leg was added to the above described neurological syndrome. The hemiplegia improved slowly but there was a gradual deterioration in his general condition due to infections (otitis media bilaterally, bronchopneumonia and urinary infection) which did not respond to treatment with ampicillin, erythromycin, nitrofurantoin or mycostatin. During the last 6 weeks of his life he had periodical septic fever. He was unconscious in the final week.

During the course of the illness the micro-ESR was about 60 mm/1 hour. The total white cell count was between 3 000 and 8 000 while the mononuclear white cells were between 700 and 1 100. There was no eosinophilia.

The preliminary autopsy showed a large abscess in the left cerebral hemisphere; otherwise there were no abnormalities visible to the naked eye in the brain, the meninges or the spinal medulla. A small area of necrosis was found in the trachea, multiple bronchopneumonic infiltrations and signs of pyelitis in the kidney. The mediastinal lymph nodes were enlarged. The thymus and the adrenals were macroscopically small. A rich growth of *Alebasella* was found in the brain abscess and on cultivation also in the blood, lungs, spleen and kidneys. After cultivation a growth of *Torulopsis glabrata* was found in the right lung and the spleen.

### REFERENCES

1. Berg R, Hansson O, Nordberg F, Sourander P, Sjöström U & Wilén R. Fatal varicella generalisata in a child with immunopathy and hereditary neurological syndrome. A report with autopsy, electron microscopy and virus isolation. *Pediatrics Arch Abt A Path Anat* 346:103, 1969.
2. Centerville S A & Centerville W R. Ataxia telangiectasia. A familial degenerative disease leading to mental retardation. A case report. *Amer J Med Defec* 71:124, 1966.
3. Cooper M D, Prierson R D A & Good R A. Delineation of the thymic and bursal lymphoid systems in the chicken. *Nature* 205:143, 1965.
4. DeGeorge A M. Congenital absence of the thymus and its immunologic consequences. Coincidence with congenital hyperparathyroidism in Bergsma & Good (eds) *Immunologic deficiency diseases in man*. Birth defect original article series vol IV no 1 p 116, 1968.
5. Odell H, Jostesky C A, Apt L & Craig J M. Agammaglobulinemia in Lawrence (ed) *Cellular and humoral aspects of the hyperimmune states*. Hoeber Harper, New York 1959 p 375.
6. Gustavsson K H, Hagberg B & Sanner G.



corresponded to the so called dys-equilibrium state (7) dominated by proprioceptive and perceptual defects and with no or only minor components of intention tremor and dysmetria.

The etiology of ataxic forms of cerebral palsy remains in most cases obscure. Prenatal factors are considered to have been at work in the majority according to Ingram (9) prenatal factors are the cause of ataxic diplegia in about 50%. In familial cases of cerebral palsy Gustavsson *et al* (6) found in a recent series that ataxic forms were much more frequent than other syndromes. The mode of inheritance in ataxic diplegia was considered to be autosomally recessive, autosomally dominant or sexlinked recessive. Our two cases are believed to belong to the first category.

In addition to their ataxic diplegia, our two siblings also showed evidence of deficient cellular immunity. To our knowledge a familial syndrome of this type has not been reported previously. There are similarities to ataxia telangiectasia (Louis Bar syndrome). However clinically the well known features of that syndrome are clearly different from those in our cases. Neither telangiectasia nor the typical cerebellar ataxia was present. In addition to qualitative differences the ataxic diplegia of our patients appeared to be non progressive, with reservation for the limited number of years of follow up. The histopathological picture in our autopsy case also differed from that in ataxia telangiectasia. In our girl the cerebellum was of normal size at autopsy while in the Louis Bar syndrome it is atrophied (2, 14, 15). In our case there was dysplasia of nerve cells in the cortex of the cerebrum and basal ganglia, and heterotopic nerve cells were found in the white matter. In the cerebellum there was a patchy loss of Purkinje cells probably dysplasia. Non specific degeneration was seen in dorsal root ganglia and in the lateral cortico spinal tract (1). These changes in the central nervous system do not tally with those described in ataxia telangiectasia (14, 15).

The deficiency in cellular immunity in the girl was proven by her failure to respond to

intracutaneous injection of vaccinia antigen in spite of the severe vaccinia gangrenosa (8).

The boy was judged to have a defect in his cellular immunity from the failure of the attempt at contact sensitization with DNCB and from the lack of response to PHA. The negative reaction to the test with candida antigen is compatible with this assumption. The system producing humoral antibodies, on the other hand seemed to be essentially normal as judged from the normal values of the various immunoglobulins. Earlier the boy showed subnormal IgG values but these were by no means comparable to the very low values seen in hypogammaglobulinemic states (5). According to the delineation of the lymphoid system presented by Cooper *et al* (3) and by Parrot *et al* (12) the germinal centers belong to the part of this system involved in antibody production. According to this view the occurrence of germinal centers in the lymph nodes of our patient thus fits with the normal immunoglobulin levels in his serum. The number of lymphocytes in the peripheral blood was low on several occasions of determination. No real aplymphopenic episodes were noted, however and it seems as if the possible deficiency in the lymphocyte production would be only moderate.

The immunological status of the patient thus showed a combination of a defect in his cellular immunity and on some occasions subnormal lymphocyte content in the peripheral blood but a fair production of immunoglobulins. A similar picture is seen in many cases of Louis Bar ataxia telangiectasia (13). Also these patients show impaired development of contact sensitivity and some of them have only minor defects in their immunoglobulin production. Further their lymph nodes contain follicular structures and often show a reticulo-endothelial hyperplasia.

From a purely immunological point of view the DiGeorge syndrome (4) must also be kept in mind. This syndrome is characterized by hypoparathyroidism and congenital absence of the thymus, normal levels of immunoglobulins

## BLOOD COAGULATION IN CHILDREN WITH CYANOTIC CONGENITAL HEART DISEASE

NILS J IOLSTER

*From the Department of Paediatric Hematology Section of Medical Department A and Institute for Thrombosis Research Rikshospitalet Oslo Norway*

awareness of the danger of operative or postoperative hemorrhagic complications of the surgical correction of congenital heart disease (4) was followed by reports of underlying deficiencies in the mechanisms of hemostasis in infants with congenital heart lesions of the cyanotic variety (8-13). Since these early studies numerous others have been published reporting a very wide variety of coagulation disturbances but there has in many instances been a noticeable lack of agreement between the published results. The present paper reports on the coagulation status of a group of patients with cyanotic heart disease and compares the results with those obtained in a control group. Since a tendency to thrombosis would appear to be at least as significant clinically as a bleeding tendency in these patients this abnormality in hemostasis is also discussed.

### MATERIAL AND METHODS

Thirty-three patients with cyanotic congenital heart disease were studied. Seventeen patients were used as controls. The criterion for the selection of the control patients was that they must not have been receiving medication, nor have conditions that could conceivably affect their hemostatic systems. For the purpose of evaluating the platelet count 19 additional patients admitted previously to the hospital for evaluation of congenital cyanotic heart disease were included in the patient group and platelet counts were performed on 30 healthy children from the child welfare clinic for inclusion in the control group.

The age distribution of the patients with cyanotic heart disease (henceforth referred to as the "patient" group) and the control patients (henceforth referred to as the "control" group) is shown in Fig. 1.

Finally the records of all patients with congenitally placed Fallot's tetralogy admitted to this hospital in the preceding 10 years were reviewed and their mean corpuscular hemoglobin (MCH) compared to that of patients with the same condition and spontaneous cerebrovascular accidents. There were 61 patients of which 8 developed ages compatible with this diagnosis.

Capillary blood was used for platelet and blood counts and for hemoglobin and hematocrit determinations. All other tests were performed on venous blood obtained through a silicone-coated needle and allowed to drip freely into 5 ml plastic test tubes containing a 0.1 M solution of sodium citrate as anticoagulant. The volume of this solution was carefully adjusted to the hematocrit. The sample was kept on melting ice until centrifuged at 3 000 r.p.m. and 4°C for 10 min. The supernatant plasma was removed with a silicone-coated pipette distributed in plastic test tubes in 1 ml aliquots and examined immediately or stored at -20°C. It was attempted to obtain 15 to 20 ml of blood from each patient but this was frequently not possible because of the size of the veins and the high viscosity. In a few patients blood was removed with a plastic syringe through a plastic catheter inserted in the cephalic vein prior to heart catheterization.

The following tests were performed on venous blood: Prothrombin proconvertin (PT) time according to the method of Owen & Aas (10) expressing the combined activity of factors II, VII and X; Cephalin (partial thromboplastin) time as described by Eggberg (7); Quick's thromboplastin time using human brain thromboplastin; Thrombin time using a commercial thrombin preparation (Hoffman-La Roche) diluted so as to give a thrombin time of approxi-

- Identical syndromes of cerebral palsy in the same family *Acta Paediat Scand* 58 330 1969
- 7 Hagberg B Sanner G & Steen M The dys-equilibrium syndrome in cerebral palsy *Acta Paediat Scand* To be published
  - 8 Hansson O Johansson S G O & Vahlquist B *Vaccinia gangrenosa* with normal humoral antibodies A case possibly due to deficient cellular immunity treated with N-methylsulfon- $\beta$ -thiosemicarbazone (Compound 33T57 Marboran) *Acta Paediat Scand* 55 264 1966
  - 9 Ingram T T S *Paediatric aspects of cerebral palsy* H & E Livingstone Ltd Edinburgh and London 1964
  - 10 Ling N R *Lymphocyte stimulation* North Holland Publishing Company Amsterdam 1968
  - 11 Nezelof C Thymic dysplasia with normal immunoglobulins and immunologic deficiency Pure lymphocytosis in D Bergsma & R A Good (eds) *Immunologic deficiency diseases in man* Birth Defect Original Article Series vol IV no 1 p 104 1968
  - 12 Parrot D M V de Sousa M A B & East J Thymus-dependent areas in the lymphoid organs of neonatally thymectomized mice *J Exp Med* 123 191 1966
  - 13 Peterson R D A Cooper M D & Good R A Lymphoid tissue abnormalities associated with ataxia telangiectasia *Amer J Med* 41 342 1966
  - 14 Sourander P Bonnevier J O & Olsson Y A case of ataxia telangiectasia with lesions in the spinal cord *Acta Neurol Scand* 47 354 1968
  - 15 Terplan K L & Krauss W F Histopathologic brain changes in association with ataxia telangiectasia *Neurology* 19 446 1969
  - 16 Thierfelder S A A method for the isolation of human lymphocytes *Vox Sangvina* 9 447 1964
  - 17 Waldorf D S Wilkens R F & Decker J L Impaired delayed hypersensitivity in an aging population *JAMA* 203 111 1968

Submitted Nov 11 1969

Accepted Febr 16 1970

(B H) Dept of Paediatrics  
Akademiska sjukhuset  
750 14 Uppsala 14  
Sweden

**Key words** Ataxia diplegia dysequilibrium cellular immunity delayed hypersensitivity 2,4-dinitrochlorobenzene phytohemagglutinin

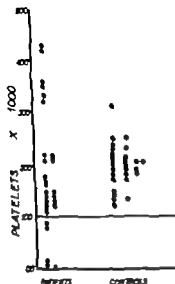


Fig 3 Platelet counts in patients and controls

was employed. It was soon evident that patients with high hematocrit could have virtually normal bleeding times despite considerable thrombocytopenia. It was assumed that bleeding times which under other circumstances would have been prolonged were being affected by the high viscosity and the test was therefore not performed by routine on these highly polycythemic patients.

**Platelet count.** Nineteen of 47 patients and 5 of 35 controls had counts below 150 000. This difference is statistically significant ( $p < 0.005$ ). Furthermore nine of the 47 patients had counts below 100 000 (Fig 3).

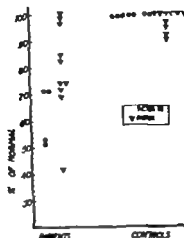


Fig 5 Factor II and VII levels in 10 patients and 10 controls

**Fibrinogen.** Plasma fibrinogen levels were within (or in isolated instances above) the normal range of 150 to 350 mg/100 ml in 31 of 32 patients and in all of the 16 controls. One patient had a level of 70 mg/100 ml. If the results were converted to whole blood fibrinogen on the basis of the hematocrit levels, 13 of 32 patients and none of the 16 controls had lower than normal values ( $p < 0.005$ ) (Fig 4).

**Other coagulation factors.** It was originally intended to measure individual coagulation factors in all patients with a prolonged cephalin time and/or clinical signs of a hemorrhagic disorder. Only 1 patient had an abnormal cephalin time. Nevertheless because of the frequency with which a prolonged prothrombin

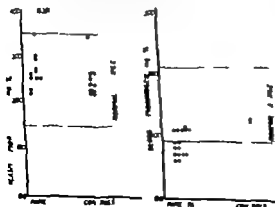


Fig 4 Plasma and whole blood fibrinogen levels in patients and controls

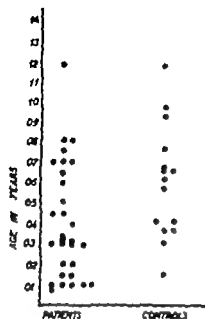


Fig 1 Age distribution of patients and controls

mately 20 sec with normal plasma. Fibrinogen concentration determined spectrophotometrically by the method of Jacobson (16) modified by Blombäck & Blombäck (5) and Gollil (11). All the coagulation tests with the exception of the thrombin time were performed in duplicate using 0.1 ml of plasma. Thrombin time was performed in a single test using 0.2 ml of plasma. Only new non-sterilized test tubes of a constant diameter were employed.

In addition to the tests mentioned above bleeding time (Ivy's method), tourniquet test, euglobulin clot lysis time and determination of isolated factors were performed on a number of patients. The method for factor II assay was the RVV cephalin method (14) and for factor VII a one stage assay using congenital factor VII-deficient substrate plasma.

## RESULTS

**Cephalin time** One patient had a significantly prolonged cephalin time. Factors VIII and V were measured in this patient and were 100% and 87% respectively. One patient had a slightly prolonged cephalin time and the remaining patients had normal values as did the controls (Fig 2).

**Combined factor II, VII and X activity** In 21 of 30 patients as opposed to 1 of 17 controls the PP method showed values below the lower limit of normal ( $p < 0.0001$ ) (Fig 2).

**Quick & thromboplastin time** In 2 of 32 patients and in 1 of 15 controls times moderately in excess of the upper limit of normal were found (Fig 2).

**Thrombin time** The thrombin dilutions employed gave thrombin times with normal plasma which ranged between 17 and 24 sec. The results are expressed as the number of seconds above (+) or below (-) the thrombin time which the particular thrombin dilution employed had given with normal plasma. No difference could be shown in the thrombin times of 26 patients compared with those of 17 controls (Fig 2).

**Bleeding time** Ivy's technique using two 1 cm long superficial incisions instead of punctures and a b.p. cuff pressure of 40 mm Hg.

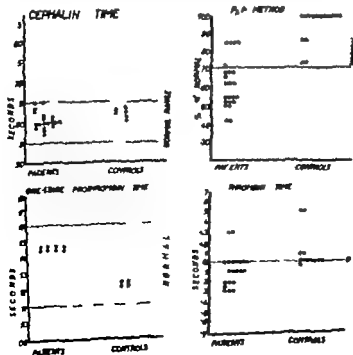


Fig 2 Cephalin time, combined II, VII and X (PP) value, Quick thromboplastin (one stage prothrombin) time and thrombin time in patients and controls.

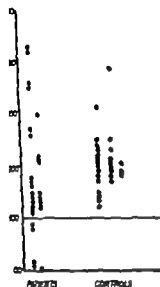


Fig. 3 Platelet counts in patients and controls

is employed. It was soon evident that patients with high hematocrits could have virtually normal bleeding times despite considerable thrombocytopenia. It was assumed that bleeding times which under other circumstances could have been prolonged were being affected by the high viscosity and the test was therefore not performed by routine on these highly polycythemic patients.

**Platelet count.** Nineteen of 47 patients and 1 of 35 controls had counts below 150 000. This difference is statistically significant ( $p < 0.005$ ). Furthermore none of the 47 patients had counts below 100 000 (Fig. 3).

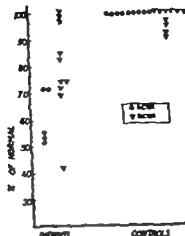


Fig. 5 Factor II and VII levels in 10 patients and 10 controls

**Fibrinogen.** Plasma (fibrinogen levels were within (or in isolated instances above) the normal range of 150 to 350 mg/100 ml in 31 of 32 patients and in all of the 16 controls. One patient had a level of 70 mg/100 ml. If the results were converted to whole blood fibrinogen on the basis of the hematocrit levels, 13 of 32 patients and none of the 16 controls had lower than normal values ( $p < 0.005$ ) (Fig. 4).

**Other coagulation factors.** It was originally intended to measure individual coagulation factors in all patients with a prolonged cephalin time and/or clinical signs of a hemorrhagic disorder. Only 1 patient had an abnormal cephalin time. Nevertheless because of the frequency with which a prolonged prothrombin

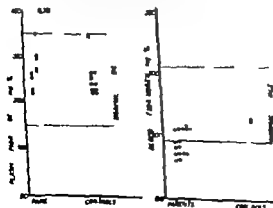


Fig. 4 Plasma and whole blood fibrinogen levels in patients and controls

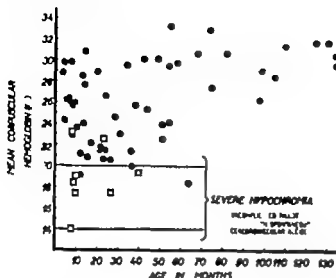


Fig 6 Mean corpuscular hemoglobin of patients with and without spontaneous cerebrovascular accidents in relation to age

proconvertin time was observed factors II and VII were determined in 10 patients with a decreased PP value and in 10 children chosen at random from the control group. A statistically significant decrease could be shown for both factors ( $p < 0.001$ ), the decrease being more marked for factor VII (Fig 5).

**Other determinations.** Euglobulin clot lysis time was determined in 4 patients and 5 controls. The values for the patients were 180, 90, 225 and 270 min and for the controls 85, 85, 85, 120 and 120 min. All these results are within the normal range.

**Analysis of case material.** Eight of the 61 patients admitted for Fallot's tetralogy had developed signs compatible with the diagnosis of spontaneous cerebrovascular accident. Six of these 8 patients had a MCH below 20, whereas only 3 of the 32 patients in the same agegroup as the former but without this complication had such a low value (Fig 6).

## DISCUSSION

The results of this study show a statistically significant difference in the concentration of factors II, VII and X using the PP method, in the concentration of fibrinogen when expressed as whole blood fibrinogen, and in the incidence of thrombocytopenia in children with cyanotic heart disease as compared to a group of children without heart disease. No difference

could be shown in the cephalin time, Quick thromboplastin time or plasma fibrinogen.

**Coagulation factors.** Reduction of a specific coagulation factor has been reported either as an isolated or as a combined deficiency for virtually all factors, but the variable and partly conflicting nature of the reports and the usually low incidence of the deficiencies described, coupled to a lack of controls (6), has made their interpretation difficult. Most studies have shown some lengthening of the Quick thromboplastin time (9, 10, 13, 15), though not to the extent originally described (8), before the need for careful adjustment of anticoagulant to the hematocrit had been demonstrated (9).

A reduction of any one of the factors involved in the cephalin time to a level of 30%–50% will prolong it (12, 22). On the basis of this test, therefore, it can be concluded that in the present patients there was no clinically significant reduction of the plasmatic factors involved in the intrinsic system, though minor reductions can not be excluded. A reduction in the combined factor II, VII and X level as measured by the PP method was established in the patient group. Determination of the individual factors in a group of patients and a group of controls showed that the difference is more pronounced for factor VII, though it is also present for factor II. Factor V levels do not influence the PP method and factor X was not measured, but these factors are com-

to both systems and a clinically significant action if any one of them should have been up as a prolonged cephalin and/or act thromboplastin time.

Liver damage has been demonstrated in dogs with hypoxemia of pulmonary origin (1) and could underlie the clinically insignificant reductions of factors II and VII found in this study.

With one exception no decrease in plasma fibrinogen was found in 32 patients. Conversion of whole blood fibrinogen values showed a reduction in almost 50% of the patients. The significance of a reduced whole blood fibrinogen in the face of a normal plasma concentration is an open question but it has been considered to be of importance (13) presumably for the mechanical qualities of the clot.

**Platelets.** Thrombocytopenia has been a frequent finding in patients with cyanotic heart disease (10, 13, 21, 27, 28) though its frequency and its relevance to the heart disorder have been questioned (1, 2, 3, 23). In this study the platelet count was below 100 000 in 9.5% of the patients. A correlation between platelet count and the arterial oxygen saturation in cyanotic heart disease has been reported (17) and thrombocytopenia has also been described in adults with lung insufficiency (18).

#### *Therapeutic considerations*

Before operative or postoperative hemorrhage in patients with congenital cyanotic heart disease operated upon without the use of extracorporeal circulation appears to be related to the age at operation, its frequency increasing with age (13). The present policy of early palliative or corrective surgery would thus appear to have largely removed this hazard. Extracorporeal circulation does of course introduce other problems of hemostasis not evaluated here. (10). Postoperative thrombosis at the site of an anastomosis or elsewhere is a more likely consequence of the altered hemostasis than hemorrhage and thus measures aimed at its prevention would be welcome.

Anticoagulants have been advised against (13) and are difficult to use in these polycythemic children. Tausig has emphasized the importance of adequate hydration (26).

Though high viscosity coupled to postoperative changes in coagulation and sometimes inadequate hydration probably plays a part in postoperative thrombosis, no correlation with a high hematocrit appears to exist for spontaneous cerebrovascular accidents (19). Thus cerebral thrombosis is commonest in the first year of life, an age in which very high hematocrit levels are uncommon. Instead there appears to be a correlation with a low MCH (19) and the findings in this study support this hypothesis (Fig. 6). The therapeutic implication is straightforward. The same age associated factors which frequently lead to iron deficiency in small children will also be acting in those with cyanotic heart disease. Their hematological status should be closely watched in infancy and early childhood and supplementary iron given if the MCH starts to decrease.

#### SUMMARY

A comparison of the coagulation status of 33 children with cyanotic congenital heart disease to that of a control group is presented. The patient group showed a statistically significant decrease in the concentration of factors II and VII and in the concentration of fibrinogen when expressed as whole blood fibrinogen and a statistically significant increase in the incidence of thrombocytopenia. No difference could be shown in the cephalin (partial thromboplastin) time, thrombin time or plasma fibrinogen concentration. Except for the thrombocytopenia, none of the deficiencies found in this study seem important enough to give rise to a hemorrhagic syndrome or to be the cause of increased operative blood loss. Evidence in the literature indicates that the risk of increased operative blood loss in cyanotic congenital heart disease is greater when the patient is an older child or adult. Present policies by which severely hypoxemic children with



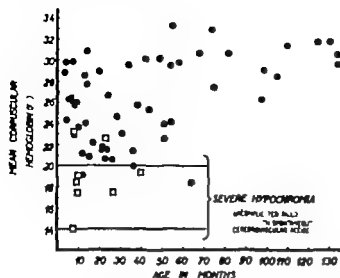


Fig 6 Mean corpuscular hemoglobin of patients with and without spontaneous cerebrovascular accidents in relation to age

proconvertin time was observed factors II and VII were determined in 10 patients with a decreased PP value and in 10 children chosen at random from the control group. A statistically significant decrease could be shown for both factors ( $p < 0.001$ ) the decrease being more marked for factor VII (Fig 5).

**Other determinations** Euglobulin clot lysis time was determined in 4 patients and 5 controls. The values for the patients were 180, 90, 225 and 270 min and for the controls 85, 85, 85, 120 and 120 min. All these results are within the normal range.

**Analysis of case material** Eight of the 61 patients admitted for Fallot's tetralogy had developed signs compatible with the diagnosis of spontaneous cerebrovascular accident. Six of these 8 patients had a MCH below 20, whereas only 3 of the 32 patients in the same agegroup as the former but without this complication had such a low value (Fig 6).

## DISCUSSION

The results of this study show a statistically significant difference in the concentration of factors II, VII and X using the PP method in the concentration of fibrinogen when expressed as whole blood fibrinogen and in the incidence of thrombocytopenia in children with cyanotic heart disease as compared to a group of children without heart disease. No difference

could be shown in the cephalin time, Quick thromboplastin time or plasma fibrinogen.

**Coagulation factors** Reduction of a specific coagulation factor has been reported either as an isolated or as a combined deficiency for virtually all factors, but the variable and partly conflicting nature of the reports and the usually low incidence of the deficiencies described coupled to a lack of controls (6) has made their interpretation difficult. Most studies have shown some lengthening of the Quick thromboplastin time (9, 10, 13, 15) though not to the extent originally described (8) before the need for careful adjustment of anticoagulant to the hematocrit had been demonstrated (9).

A reduction of any one of the factors involved in the cephalin time to a level of 30%–50% will prolong it (12, 22). On the basis of this test therefore it can be concluded that in the present patients there was no clinically significant reduction of the plasmatic factor involved in the intrinsic system though minor reductions can not be excluded. A reduction in the combined factor II, VII and X level as measured by the PP method was established in the patient group. Determination of the individual factors in a group of patients and a group of controls showed that the difference is more pronounced for factor VII, though it is also present for factor II. Factor V level does not influence the PP method and factor X was not measured but these factors are com-

to both systems and a clinically significant action of any one of them should have shown up as a prolonged cephalin and/or axt thromboplastin time.

Liver damage has been demonstrated in rats with hypoxemia of pulmonary origin (1) and could underlie the clinically insignificant reductions of factors II and VII found in this study.

With one exception, no decrease in plasma prothrombin was found in 32 patients. Conversion of whole blood fibrinogen values showed a reduction in almost 50% of the patients. The significance of a reduced whole blood fibrinogen in the face of a normal plasma concentration is an open question but it has been considered to be of importance (13) presumably for the mechanical qualities of the clot.

**Platelets.** Thrombocytopenia has been a frequent finding in patients with cyanotic heart disease (10, 13, 21, 27, 28) though its frequency and its relevance to the heart disorder has been questioned (1, 2, 3, 23). In this study the platelet count was below 100 000 in 75% of the patients. A correlation between platelet count and the arterial oxygen saturation in cyanotic heart disease has been reported (17) and thrombocytopenia has also been described in adults with lung insufficiency (18).

#### *Therapeutic considerations*

Continuous operative or postoperative hemorrhage in patients with congenital cyanotic heart disease operated upon without the use of extracorporeal circulation appears to be related to the age at operation, its frequency increasing with age (13). The present policy of early palliative or corrective surgery would thus appear to have largely removed this hazard. Extracorporeal circulation does of course introduce other problems of hemostasis not evaluated here (10). Postoperative thrombosis at the site of an anastomosis or elsewhere is a more likely consequence of the altered hemostasis than hemorrhage and thus measures aimed at its prevention would be welcome.

Anticoagulants have been advised against (13) and are difficult to use in these polycythemic children. Tausig has emphasized the importance of adequate hydration (26).

Though high viscosity coupled to postoperative changes in coagulation and some times inadequate hydration probably plays a part in postoperative thrombosis, no correlation with a high hematocrit appears to exist for spontaneous cerebrovascular accidents (19). Thus cerebral thrombosis is commonest in the first year of life, an age in which very high hematocrit levels are uncommon. Instead there appears to be a correlation with a low MCH (19) and the findings in this study support this hypothesis (Fig. 6). The therapeutic implication is straightforward. The same age associated factors which frequently lead to iron deficiency in small children will also be acting in those with cyanotic heart disease. Their hematological status should be closely watched in infancy and early childhood and supplementary iron given if the MCH starts to decrease.

#### SUMMARY

A comparison of the coagulation status of 33 children with cyanotic congenital heart disease to that of a control group is presented. The patient group showed a statistically significant decrease in the concentration of factors II and VII and in the concentration of fibrinogen when expressed as whole blood fibrinogen and a statistically significant increase in the incidence of thrombocytopenia. No difference could be shown in the cephalin (partial thromboplastin) time, thrombin time or plasma fibrinogen concentration. Except for the thrombocytopenia, none of the deficiencies found in this study seem important enough to give rise to a hemorrhagic syndrome or to be the cause of increased operative blood loss. Evidence in the literature indicates that the risk of increased operative blood loss in cyanotic congenital heart disease is greater when the patient is an older child or adult. Present policies by which severely hypoxemic children with

congenital heart disease are submitted to palliative or corrective procedures at an early age should minimize the danger of hemorrhage. Nevertheless a careful investigation of the coagulation status of all patients with cyanotic congenital heart disease who are to undergo surgical procedures is indicated. Thrombosis, especially cerebral thrombosis, is a more important complication than hemorrhage in the pediatric age group. Evidence is presented in support of the view that iron deficiency predisposes to this complication possibly by increasing tissue hypoxia.

### ACKNOWLEDGEMENTS

The author wishes to thank Professor D. F. Hjort, Professor M. Seip and Mr M. Jeremic for valuable help and criticism. This study was supported by a grant from the Norwegian Council on Cardiovascular Diseases.

### REFERENCES

- Aligle D, Heim de Balsac H, Guery J, Pascaleq J, Blonderu P & Dubost Ch. Les thrombopathies associées aux cardiopathies congénitales. *Rev. Franc. Etud. Clin. Biol.* 3: 332, 1958.
- Aligle D & Goffaux J. Le temps de saignement au cours des cardiopathies congénitales. *Nouv. Rev. Franc. Hemat.* 3: 327, 1963.
- Les troubles préopératoires de l'hémostase dans les cardiopathies congénitales. *Nouv. Rev. Franc. Hemat.* 5: 23, 1965.
- Binsson H T & Ziegler R F. A consideration of the causes of death following operation for congenital heart disease of the cyanotic type. *Surg. Gynec. Obstet.* 90: 60, 1950.
- Blomback B & Blomback M. Purification of human and bovine fibrinogen. *Arkiv for Kemi* (Stockholm) 10: 415, 1956.
- Burrow L, Watson D G & Bell W N. Blood coagulation studies in children with congenital heart disease. *Amer. Heart J.* 70: 747, 1965.
- Egeberg O. Assay of antihemophilic A II and C factors by one stage cephalin systems. *Scand. J. Clin. Lab. Invest.* 13: 140, 1961.
- Favre Gilly J, Bret J & Borel Milhet J. Un trouble inattendu de la coagulation dans la maladie bleue: l'hypoprothrombinémie. *Sang.* 22: 278, 1951.
- Favre Gilly J. Les difficultés d'estimation du taux de la prothrombine chez les polyglobuliques. *Sang.* 26: 788, 1955.
- Gans H & Krivit W. Problems in hemostasis during and after open heart surgery VI. Over all changes in blood coagulation mechanisms. *JAMA* 179: 145, 1962.
- Godal H C. Simple syneresis procedure for fibrinogen assay. *Scand. J. Clin. Lab. Invest.* 13: 530, 1961.
- Gonyea L M, Hjort P & Owren P A. Study of relationship of concentrations of prothrombin, proconvertin and proaccelerin to 3 methods for measuring prothrombin time. *J. Lab. Clin. Med.* 48: 624, 1956.
- Hartmann R C. A hemorrhagic disorder occurring in patients with cyanotic congenital heart disease. *Bull. Johns Hopkins Hosp.* 91: 49, 1957.
- Hjort P, Rapaport S I & Owren P A. A simple specific one stage prothrombin assay using Russell's viper venom in cephalin suspension. *J. Lab. Clin. Med.* 46: 89, 1955.
- Jackson D P. Hemorrhagic diathesis in patients with cyanotic congenital heart disease. *Ann. NY Acad. Sci.* 115: 235, 1964.
- Jacobsson K. Studies on determination of fibrinogen in human blood plasma. *Scand. J. Clin. Lab. Invest.* 7: Suppl. 14, 1955.
- Kochhuber B & Gutheil H. Gerinnungsstörungen bei Kindern mit angeborenen Herzfehlern. *Z. Kreislaufforsch.* 54: 738, 1965.
- Kummer H, Gurtner H P & Bucher U. Thrombocytopenie bei chronischen Cor pulmonale und cyanotischen Herzfehlern. *Schweiz. Med. Wochschr.* 94: 1334, 1964.
- Martelle R R & Linde L M. Cerebrovascular accidents with tetralogy of Fallot. *Amer. J. Dis. Child.* 101: 206, 1961.
- Owren P A & Aas K. The control of diurnal therapy and the quantitative determination of prothrombin and proconvertin. *Scand. J. Clin. Lab. Invest.* 3: 201, 1951.
- Paul M H, Currimbhoy Z, Miller R A & Schulman I. Thrombocytopenia in cyanotic congenital heart disease. *Amer. J. Dis. Child.* 102: 597, 1961.
- Proctor M R & Rapaport S I. The partial thromboplastin time with kaolin. *Amer. J. Clin. Path.* 36: 212, 1961.
- Riby C. Les troubles de l'hémostase dans les cardiopathies congénitales: leur retentissement sur les pertes sanguines post opératoires. *Hémostasie* 2: 279, 1962.
- Refsum H B. Arterial hypoxaemia serum activities of GOT, GPT and LDH and centrilobular liver cell necrosis in pulmonary insufficiency. *Clin. Sci.* 25: 369, 1963.
- Rudolph A M, Nadas A S & Borges W H. Hematologic adjustments to cyanotic congenital heart disease. *Pediatrics* 11: 454, 1953.
- Tausig H H. *Congenital malformations of the heart*. 2nd ed. Commonwealth Fund, New York, 1960.

- 17 van Creveld S Hemorrhagic diathesis in congenital heart disease. Influence of operation under hypothermia and of whole blood transfusions *Ann Paediatr* 190 3-42 1958
- 18 Veral D, Mazurino S J, Blackburn E B, Izery J L, Varadi S & Wolman L Thrombocytopenia in congenital heart disease *Brit Heart J* 24 92 1967

Submitted September 18 1969

Accepted March 15 1970

Departamento de Pediatría  
Hospital de Clínicas  
Córdoba 2149  
Buenos Aires  
Argentina

*Key words* Congenital cyanotic heart disease bleeding tendency blood coagulation thrombocytopenia thrombotic episodes iron deficiency

congenital heart disease are submitted to palliative or corrective procedures at an early age should minimize the danger of hemorrhage. Nevertheless a careful investigation of the coagulation status of all patients with cyanotic congenital heart disease who are to undergo surgical procedures is indicated. Thrombosis, especially cerebral thrombosis, is a more important complication than hemorrhage in the pediatric age group. Evidence is presented in support of the view that iron deficiency predisposes to this complication possibly by increasing tissue hypoxia.

### ACKNOWLEDGEMENTS

The author wishes to thank Professor P. F. Hjort, Professor M. Seip and Mr. M. Jeremic for valuable help and criticism. This study was supported by a grant from the Norwegian Council on Cardiovascular Diseases.

### REFERENCES

1. Aligalle D, Heim de Balzac R, Guery J, Puaulecq J, Blondelet P & Dubost Ch. Les thrombopathies associées aux cardiopathies congénitales. *Rev Franc Etud Clin Biol* 3:332 1958
2. Aligalle D & Goffaux J. Le temps de saignement au cours des cardiopathies congénitales. *Ann Rev Franc Hemat* 3:327 1963
3. — Les troubles préopératoires de l'hémostase dans les cardiopathies congénitales. *Novi Rev Franc Hemat* 5:23 1965
4. Bahnsen H T & Ziegler R F. A consideration of the causes of death following operation for congenital heart disease of the cyanotic type. *Surg Gynec Obstet* 90:60 1950
5. Blombäck B & Blombäck M. Purification of human and bovine fibrinogen. *Arkiv for Kemi (Stockholm)* 10:415 1956
6. Burton L, Watson D G & Bell W N. Blood coagulation studies in children with congenital heart disease. *Amer Heart J* 70:747 1965
7. Egeberg O. Assay of antithrombin A B and C factors by one stage cephalin systems. *Scand J Clin Lab Invest* 13:140 1961
8. Favre Gilly J, Bret J & Borel Milhet J. Un trouble méconnu de la coagulation dans la maladie bleue l'hypoprothrombinémie. *Sang* 22:278 1951
9. Favre Gilly J. Les difficultés d'estimation du taux de la prothrombine chez les polyglobuliques. *Sang* 26:788 1955
10. Gaus H & Krivit W. Problems in hemostasis during and after open heart surgery. VI. On all changes in blood coagulation mechanisms. *AMA* 179:145 1962
11. Godal H C. Simple syntheses procedure for fibrinogen assay. *Scand J Clin Lab Invest* 11:530 1961
12. Gonyea L M, Hjort P & Owren P A. Study of relationship of concentrations of prothrombin, proconvertin and proaccelerin to 3 methods for measuring prothrombin time. *J Lab Clin Med* 48:623 1956
13. Hartmann R C. A hemorrhagic disorder occurring in patients with cyanotic congenital heart disease. *Bull Johns Hopkins Hosp* 91:49 1957
14. Hjort P, Rapaport S I & Owren P A. A simple specific one stage prothrombin assay using Russell's viper venom in cephalin suspension. *J Lab Clin Med* 46:89 1955
15. Jackson D P. Hemorrhagic diathesis in patients with cyanotic congenital heart disease. *Ann NY Acad Sci* 115:235 1964
16. Jacobson K. Studies on determination of fibrinogen in human blood plasma. *Scand J Clin Lab Invest* 7 Suppl 14 1955
17. Kornhuber H & Gutheil H. Gerinnungsstörungen bei Kindern mit angeborenen Herzfehlern. *Z Kreislaufforsch* 54:718 1965
18. Kummer H, Gurtner H P & Becker U. Thrombocytopenie bei chronischen Cor pulmonale und cyanotischen Herzfehlern. *Schweiz Med Wochschr* 94:1334 1964
19. Martelle R R & Lynde L M. Cerebrovascular accidents with tetralogy of Fallot. *Amer J Dis Child* 101:206 1961
20. Owren P A & Aas K. The control of diurnal therapy and the quantitative determination of prothrombin and proconvertin. *Scand J Clin Lab Invest* 3:201 1951
21. Paul M H, Curranboy Z, Miller R A & Schulman I. Thrombocytopenia in cyanotic congenital heart disease. *Amer J Dis Child* 107:597 1961
22. Proctor R R & Rapaport S I. The partial thromboplastin time with kaolin. *Amer J Clin Path* 36:212 1961
23. Raby C. Les troubles de l'hémostase dans les cardiopathies congénitales leur retentissement sur les pertes sanguines post opératoires. *Hémostase* 2:279 1962
24. Refsum H E. Arterial hypoxaemia serum activities of GO-T, GPT and LDH and centrilobular liver cell necrosis in pulmonary insufficiency. *Clin Sci* 25:469 1963
25. Rudolph A M, Nadas A S & Borges W H. Hematologic adjustments to cyanotic congenital heart disease. *Pediatrics* 11:454 1953
26. Taussig H B. *Congenital malformations of the heart*. 2nd ed. Commonwealth Fund, New York 1960

Table 1 Clinical data on 8 patients with solitary mastocytoma

Case	Site of lesion	Age of onset	Darier's sign	Bleeding Venous lation	General symptoms	Co-existent disease	Family history	Signs of systemic involvement
1	Left thigh	Birth	+	+	Flush	-	Urticaria	-
2	Trunk	Two weeks	+	-	Flush	-	Bronchial asthma Allergic rhinitis	-
3	Left forearm	One week	+	-	-	Obstructive bronchitis	Bronchial asthma	-
4	Left forearm	Birth	+	-	Respiratory wheezing	Recurrent obstructive bronchitis	Bronchial asthma	-
5	Left forearm	Nine months	+	+	-	-	-	- (?)
6	Right forearm	Eight months	+	-	-	Neonatal hypoglycaemia Convulsive disorder	-	-
7	Sole of right foot	Birth	(-)	+	-	-	-	-
8	Left forearm	Two months	+	-	-	-	?	-

erythema which lasted for 10-15 min. The child's general condition was good.

Neither the liver nor the spleen were enlarged. The left thigh showed a 10 by 22 mm sharply outlined slightly raised plaque (Fig. 1). Darier's sign was positive (irritation of the tumour after mechanical stimulation). Vesicles formed from time to time on the surface of the skin lesion but no bullae appeared during the child's stay in hospital at 3 months of age and stroking of the lesion no longer produced generalized erythema.

The blood picture was normal. There was 0.2 basophils and 0.5 eosinophils per 100 white blood cells (1 000 cells counted). The number of bone marrow cells stained metachromatically by toluidine blue was not increased.

Histologically a very dense subepidermal aggregation of mast cells was seen. The cells had vesicular pale nuclei and fairly scanty cytoplasm which stained strongly with toluidine blue.

#### Case 4

A boy aged 10 months. His grandmother had bronchial asthma. The boy had recurrent obstructive bronchitis since six months of age. Physical examination caused wheezing.

Since birth he had had an 11 by 20 mm light brown and somewhat elevated and indurated tumour on his left forearm. Darier's sign was positive and the erythema involved a considerable portion of the forearm (Fig. 2). On one occasion a typical attack of bronchial asthma without concomitant general flushing was provoked by gentle scratching of the tumour.

The attack was curtailed by epinephrine and prednisolone.

The liver and spleen were not palpably enlarged. The blood picture was normal. Only a few meta-chromatic cells were found in the toluidine blue stained bone marrow smear.

Histologically the upper part of the dermis contained numerous groups of regular cells with light oval nuclei and rather abundant cytoplasm. The cells were arranged in columns and around blood vessels as well as in scattered groups between collagenous fibres in the deep layers of the dermis. The cytoplasmic granules were demonstrated by staining with toluidine blue and Giemsa stain.

After excision of the tumour the patient continued to have attacks of asthmatic bronchitis. The operation wound healed with keloid formation.

Fig. 3 shows the histological appearance in case no. 2.

## RESULTS

The results of the laboratory studies are summarized in Table 2. The 24 hour urinary excretion of histamine was determined in five of the children. It was normal in four but substantially increased in one. This patient had generalized flushes though not during the period of collection of the urine. The histamine values found in this patient are given in Table

## SOLITARY MASTOCYTOMA

LARS HOLMBERG

From the Department of Paediatrics Malmö General Hospital Malmö Sweden

Mastocytosis is a term used to denote a pathological infiltration of mast cells in the skin or other tissues. It may be divided into a *cutaneous* and a *systemic* form. Three types of the cutaneous form may be recognized with regard to the extent of the disease: *solitary mastocytoma*, *disseminated* and *diffuse mastocytosis* (34). The disseminated type of cutaneous mastocytosis corresponds to the form of urticaria pigmentosa originally described in 1869 (27).

In systemic mastocytosis infiltrations of mast cells are present in such extra-cutaneous sites as bone and bone marrow, liver, spleen, lymph nodes. The condition may be fatal even in childhood. Much of the interest in mastocytosis is due to the fact that mast cells contain and elaborate substances responsible for many of the manifestations of the disease (21).

*Solitary mastocytoma* in man was first described in 1934 (17). Mastocytoma is a common tumour in dogs (4) and also occurs in other animals (30). Some 75 cases have so far been recorded in man (6, 7, 9, 10, 14, 19, 20).

This paper reports eight cases of solitary mastocytoma seen within a three year period at the department of paediatrics in Malmö General Hospital.

### MATERIAL

The series consisted of eight children, aged 5-18 months, with solitary mastocytoma. Judging from skeletal X-ray and haematological and bone marrow studies the disease was not systemic. Six of the children were seen by the author. A search of the records of various clinical departments of the hospital

revealed two additional cases of solitary mastocytoma and six cases of disseminated urticaria pigmentosa in such young children. The present material was confined to the patients with isolated lesions (this type of the disease having received less attention in the literature). Clinical data on the eight cases are presented in Table 1.

### METHODS

The excretion of histamine in the urine was estimated in five cases. In four the amine was measured by bio-assay on isolated guinea pig ileum (37). In one case (no. 1) both the histamine and the methylhistamine were estimated and then by a spectrophotometric method (16).

As a rule coagulation studies included coagulability time and recalcification time of citrated plasma, bleeding time (Duke & Ivy), thrombocytes, one stage prothrombin time with human brain thromboplastin (Quick), P & P (31), factor V (38), fibrinogen determination and estimation of fibrinolysis on fibrin plates and euglobulin clot lysis time (28) and estimation of circulating anticoagulant (the effect of the patient's plasma on the coagulation time of normal blood and on the recalcification time and prothrombin time of normal plasma, thrombin titration (29)). In one patient (no. 5) the local fibrinolysis in the area of the tumour was tested with a modification of Todd's fibrin slide method (32).

### CASE REPORTS

Two cases are described in some detail (no. 1 and no. 4).

#### Case 1

A girl, aged 5 months. Her mother had urticaria. The child had had a skin lesion on her left thigh since birth. At 2 1/2 months a blister had appeared on the surface of the lesion. The blister had persisted for 14 days and then dried up without rupturing. At 4 months rubbing of the tumour induced generalized



Fig 2 Solitary mastocytoma on left forearm. Positive Darier's sign. Erythema covers major part of forearm.

s mutated form can hardly pass unnoticed and practically all cases of the solitary variant with its fairly conspicuous symptoms must be detected though the lesions may sometimes be mistaken for a common nevus or some other skin disease. A positive Darier's sign was the clue to the diagnosis in all 8 cases. But this sign is not conclusive since a few other cases with a suspected Darier's sign were observed where biopsy failed to confirm the diagnosis. Yet, it must be borne in mind that even histological examination may sometimes miss mast cells undergoing degranulation.

The tumour appeared as a macule in one case as a papule in one as a small nodule in one and in the remaining 5 as a more or less raised plaque. The lesion was noticed at or

soon after birth in six of the infants but not until 8 and 9 months after birth in the remaining two.

In these respects the lesions resembled those on record. Solitary mastocytoma is apparently more often situated on the limbs especially on the forearm and around the wrist than on the trunk (9-34). In one published case the tumour was situated in the sole of the foot (1) as in case 7. Mastocytoma is as a rule observed at birth or during the first few weeks or months of life but it may occur later even in adult age (19).

No cases of mastocytosis were known among any of the other members of the families. The material thus lent no support to the opinion that mastocytosis is a genetic disease (35). On





Fig 1 Solitary mastocytoma. Sharply demarcated and slightly raised reddish plaque on left thigh.

3. There may have been a loss of urine during the sampling as the volumes were small. A 24 hour sample was collected first. The next day the urine was collected in two portions: one 8 hour sample and one 16 hour sample. The 8 hour sample was collected during the day when the patient was given aspirin and when the tumour was reportedly stroked.

### DISCUSSION

Eight cases of solitary mastocytoma have occurred among children born in the city of Malmö in 1966-1968 which means an incidence of 0.7 per thousand. In addition six cases of disseminated urticaria pigmentosa have been dis-

covered among the children born in these years. Both forms of mastocytosis had invariably appeared before the children were one year of age. The total incidence was 1.3 per thousand.

Thus in the present study solitary mastocytoma does not seem to be so rare as might be assumed from the literature. Contrary to what might have been expected from the common notion of the relative incidences the solitary variant even outnumbered the disseminated form of the disease. Solitary mastocytoma has been described as constituting 10-15% of all cases of mastocytosis (19). No reliable figures are available on the total incidence of mastocytosis. The incidence reported here presumably comes close to the true incidence. The dis-



Fig. 2 Solitary mastocytoma on left forearm. Positive Darier's sign. Erythema covers major part of forearm.

a mutated form can hardly pass unnoticed, and practically all cases of the solitary variant with its fairly conspicuous symptoms must be detected though the lesions may sometimes be mistaken for a common nevus or some other skin disease. A positive Darier's sign was the clue to the diagnosis in all 8 cases. But this sign is not conclusive since a few other cases with a suspected Darier's sign were observed where biopsy failed to confirm the diagnosis. Yet it must be borne in mind that even histological examination may sometimes miss mast cells undergoing degranulation.

The tumour appeared as a macule in one case, as a papule in one, as a small nodule in one, and in the remaining 5 as a more or less raised plaque. The lesion was noticed at or

soon after birth in six of the infants but not until 8 and 9 months after birth in the remaining two.

In these respects the lesions resembled those on record. Solitary mastocytoma is apparently more often situated on the limbs, especially on the forearm and around the wrist than on the trunk (9, 34). In one published case the tumour was situated in the sole of the foot (1) as in case 7. Mastocytoma is as a rule observed at birth or during the first few weeks or months of life but it may occur later even in adult age (19).

No cases of mastocytosis were known among any of the other members of the families. The material thus lent no support to the opinion that mastocytosis is a genetic disease (35). On

the other hand as in Caplan's series (9) the observed frequency of allergic disorders among such members was higher than that expected

Two patients had general symptoms. One patient exhibited a general flush precipitated by mechanical irritation of the lesion. This patient had an increased urinary excretion of histamine. General flushing is characteristic of urticaria pigmentosa and also of the solitary variant (2, 3, 7, 12, 23, 25). The flush is generally believed to be induced by the release of histamine into the circulation from the tumour.

Cases of urticaria pigmentosa with bronchial asthma are known (24). Histamine can produce bronchial constriction in individuals with bronchial asthma or bronchitis but has only negligible effect on the bronchioles in normals (11).

Table 2 Results of laboratory investigation

Case	Histamine excretion $\mu\text{g/kg}$ body weight/24 h	5 HIAA excretion $\text{mg/g}$ creatinine	Coagulation studies
1	8	17.9	Normal
2	0.4	0.3 $\text{mg}/24 \text{ h}$	Normal
3	—	21.4	Normal
4	0.3-0.4	2 $\text{mg}/24 \text{ h}$	Normal
5	0.4-0.5	8.2, 14.3	Fibrinogen 0.13 g/100 ml. No local fibrinolysis in tumour
6	0.4-0.5	6.5	Normal

Table 3 Results of histamine studies in case no. 1 (spectrophotometric method)

Time (h)	Collected volume (ml)	Histamine in collected volume ( $\mu\text{g}$ )	Methylhistamine in collected volume ( $\mu\text{g}$ )
24	145	54	161
8	30	16	29
16	125	35	66



Fig. 3 Collections of mast cells in upper dermis with concentrations around excretory ducts of sweat glands and around hair follicles. Cells contain numerous metachromatic granules in sections stained with toluidine blue (case no. 2). Toluidine  $\times 75$  (Alf Rasmussen).

One patient (no. 4) who had expiratory wheezing on stimulation of the tumour obviously had a disposition to asthmatic disease.

One of the patients in the material had an apparently coincidental convulsive disorder. Such cases have also been described earlier (22). Of Coles' (10) fourteen patients with urticaria pigmentosa two had seizures without apparent causal connection. Seizures in an adult with mast cell infiltration of the meninges as a part of systemic mastocytosis have been reported (26). In this case nothing suggested such an event. The histamine shock of urticaria pigmentosa has been associated with loss of consciousness but not with seizures (5, 33).

The risk of systemic involvement is much less when mastocytosis appears in childhood than when it does not occur until later in life (34). Particularly the diffuse or bullous forms are prone to be associated with internal disturbances (8). In none of the present cases of solitary mastocytoma did clinical examination, skeletal X-ray or investigation of the bone marrow reveal any signs of systemic involvement. Visceral and skeletal disturbances have been

suspected in solitary mastocytosis (7-15) but have not been demonstrated with certainty.

Increased amounts of histamine in the urine have been found in most but not in all patients with urticaria pigmentosa. The histaminuria is probably due to overproduction of histamine by the increased number of mast cells in mastocytosis and not to an increased rate of histamine formation from histidine or to impaired catabolism of histamine (13).

Only two cases of solitary mastocytoma seem to have been studied with reference to histamine excretion and in both it was increased (7-14). In the present investigation it was normal in four cases and substantially increased in one (Tables 2 and 3). Normal values for methylnorhistamine are lacking but they are probably 10 times as high as the corresponding values for histamine (about  $0.3 \mu\text{g/kg/24 hours}$ ) (36). Anyway there is reason to assume that the values in case 1 were substantially increased. The increase cannot be explained by the use of the spectrophotometric method though it does tend to yield higher values. Nor can it be explained on dietary grounds (16). At least in cases 1-4 and 5 the tumour was of similar appearance and size and none of these children showed any evidence of internal lesions. It is therefore difficult to explain the variation in histamine excretion. There was no further increase of the histamine concentration in urine collected during mechanical stimulation of the tumour and administration of aspirin to the patient.

The urinary excretion of 5-HIAA was normal (in controls it ranged from 8-40  $\text{mg/g creatinine}$ ).

A tendency to haemorrhage is a well known but not very common characteristic of urticaria pigmentosa. It has been difficult however to correlate the bleeding tendency with the results of studies of the coagulation mechanism (18). In the present series of solitary mastocytoma laboratory studies showed no disturbances of the coagulation mechanism and particularly no abnormal amounts of a circulating anticoagulant of heparin type. Neither was there any

signs of local fibrinolytic activity in the tumour and the general fibrinolytic activity in the circulation was normal.

According to the literature the prognosis is good and in most cases the condition disappears spontaneously (9-19-20). Though the outlook is thus bright the tumour has often been a matter of great concern to the mother. Such concern can however be dispelled by early recognition of the condition and reassurance of the parents of the innocuous nature of the lesion.

### SUMMARY

Eight cases of solitary mastocytoma among children born in 1966-1968 in the city of Malmö have been recorded. Six of them were submitted to investigation. The diagnosis was confirmed by histological examination. In most cases the tumour appeared at birth or soon after. No signs of systemic mastocytosis were revealed by X-ray of the skeleton or by bone marrow studies.

One patient had an attack of generalized flushing and another had an attack of bronchial asthma precipitated by mechanical stimulation of the tumour. In the first case there was an increased urinary excretion of histamine while it was normal in all the others. The coagulation mechanism was normal and particularly no pathological amounts of a circulating anticoagulant of heparin type were demonstrated.

### ACKNOWLEDGEMENTS

The coagulation studies were performed at the Coagulation Laboratory, Malmö General Hospital (prof. I. M. Nilsson) and the histamine studies at the Chemical Physiological Laboratory, University of Lund (prof. H. Westberg).

### REFERENCES

1. Bigelow E. L. Changing concepts concerning mastocytosis. *J. Pediatr.* 58: 499, 1961.
2. Bart A. R. & Nickerson M. Generalized flushing of the skin with urticaria pigmentosa. *Arch. Derm.* 80: 311, 1959.
3. Blauch W. Solitaires tuberoses Mastocytom mit generalisierter Gefässkrone. *Z. Haut Geschlechtskr.* 28: 326, 1960.
4. Bloom F. Spontaneous solitary and multiple mast cell tumours (mastocytoma) in dogs. *Arch. Path.* 33: 661, 1942.

- 5 Bloom G, Dunér H, Pernow B, Winberg J & Zetterström R Spontaneous histamine shocks in urticaria pigmentosa *Acta Paediatr* 47 152 1958
- 6 Brett E M, Ong B H & Friedmann T Mast cell disease in children *Brit J Derm* 79 197 1967
- 7 Brogren N, Dunér H, Hamrin B, Pernow B, Theander G & Wahlenström J Urticaria pigmentosa (mastocytosis). A study of nine cases with special reference to the excretion of histamine in urine *Acta Med Scand* 163 223 1959
- 8 Burgoon C F Jr, Graham J H & McCaffree D L Mast cell disease *Arch Derm* 98 590 1968
- 9 Caplan H M The natural course of urticaria pigmentosa *Arch Derm* 87 146 1963
- 10 Cole A R C Mastocytosis Report of a case in a 15 1/2-week old infant with cutaneous and generalized skeletal changes *Arch Dis Child* 40 677 1965
- 11 Curry J J The action of histamine on the respiratory tract in normal and asthmatic subjects *J Clin Invest* 25 785 1946
- 12 Degos R, Delort J, Verhaeg J & Civatte F Mastocytose: a plaque unique du nourisson. Poursées bulleuses locales associées à une vasodilatation généralisée des téguments *Bull Soc Franc Derm Syph* 62 135 1955
- 13 Demis D J The mastocytosis syndrome Clinical and biological studies *Ann Intern Med* 59 194 1963
- 14 Demis D J, Walton M D & Higdon R S Histaminuria in urticaria pigmentosa and the mastocytosis syndrome A clinical study and review of the recent literature with definition of the mastocytosis syndrome *Arch Derm* 83 127 1961
- 15 Graciansky M P, Loewen Lyon, Grupper C & Trieb M Mastocytome unique avec pousse bulleuse *Bull Soc Franc Derm Syph* 61 104 1954
- 16 Gruner G Urinary excretion of histamine, methylhistamine and methylimidazoleacetic acid in man under standardized dietary conditions *Scand J Clin Lab Invest* 22 Suppl 104 59 1968
- 17 Gross P Urticaria pigmentosa (solitary lesion) *Arch Derm* 29 451 1934
- 18 Johansen L & Künzer W Zum Verhalten der Blutgerinnung bei Urticaria Pigmentosa *Arch Kinderheilk* 174 133 1966
- 19 Johnson W & Helwig E Solitary mastocytosis *Arch Derm* 84 806 1961
- 20 Klaus S N & Winkelmann R K Course of urticaria pigmentosa in children *Arch Derm* 86 68 1962
- 21 Lindell S E, Korsman H & Westling H Histamine formation in urticaria pigmentosa *Acta Dermatovenereol* 41 277 1961
- 22 Lynch F Urticaria pigmentosa (formerly bullous) accompanied by transient urticaria *Arch Derm* 71 668 1955
- 23 Marshall J, Walker J, Lurie H I, Hamen J D L & Mackenzie D Solitary mastocytoma and the mastocytoses: a discussion of the mastocytoses and a report of two cases of solitary mastocytoma showing an unusual phenomenon of generalized flushing *S Afr Med J* 31 867 1957
- 24 McKee W D, Cochrane C G & Farr R S A clinical study of an unusual case of asthma associated with urticaria pigmentosa *J Allerg* 37 38 1966
- 25 Morgan H, Gross H & Reisman R Solitary urticaria pigmentosa with systemic symptoms *Arch Derm* 88 473 1963
- 26 Mutter R D, Tannenbaum M & Ullmann J E Systemic mast cell disease *Ann Intern Med* 59 887 1963
- 27 Nettleship E Rare forms of urticaria *Brit med J* 18 323 1869
- 28 Nilsson I M & Olow B Determination of fibrinogen and fibrinolytic activity *Thromb Diath Haemorrhag* 8 297 1962
- 29 Nilsson I M & Wenckert A Demonstration of a heparin like anticoagulant in normal blood *Acta Med Scand Suppl* 298 1954
- 30 Orkin M Mastocytosis in animals *Arch Derm* 96 391 1967
- 31 Owen P A & Ans K The control of thrombolytic therapy and the quantitative determination of prothrombin and proconvertin *Scand J Clin Lab Invest* 3 201 1951
- 32 Pandolfi M, Isacson S, Nilsson I M & Robertson H Fibrinolytic activity of human veins *Lancet* 2 127 1967
- 33 Robinson H M, Kile R L, Hitch J M, Raikigh N C & Robinson R C V Bullous urticaria pigmentosa *Arch Derm* 85 346 1962
- 34 Sigler F & Even Paz Z *Mastocytosis and the mast cell hager* Basel and New York 1967
- 35 Shaw J M Genetic aspects of urticaria pigmentosa *Arch Derm* 97 137 1968
- 36 Westling H Evaluation of histamine metabolism in man *Scand J Clin Lab Invest* 1 23 1969
- 37 Wetterqvist H & White T Urinary excretion of histamine and methylhistamine in male and female rats *Scand J Clin Lab Invest* 22 Suppl 104 3 1968
- 38 Wolf P A modification for routine laboratory use of Stefanakis method of estimating factor V activity in human oxidized plasma *J Clin Path* 6 34 1953

Submitted Dec 12 1969

Accepted Jan 13 1970

Dept of Paediatrics  
Malmö Allmänna Sjukhus  
214 01 Malmö  
Sweden

Key words: Mastocytoma infants, histaminuria

## ALTERATIONS IN THE SLEEP PATTERNS OF INFANTS AND YOUNG CHILDREN FOLLOWING ACUTE HEAD INJURIES

H. G. LENARD and H. PENNIGSTORFF

*From the Department of Paediatrics University of Göttingen, Göttingen, West Germany*

Whereas sleep—(frequently drug induced)—is widely used in clinical electroencephalography as a method of provoking paroxysmal activity in certain epileptic patients the continuous recording by polygraphic techniques of several hours of normal infant sleep is a new tool for the investigation of variety of pathologic conditions. Both qualitative and quantitative changes in the different stages of sleep may be shown thus revealing alterations in the structures responsible for the cyclic changes of brain functions.

In patients with severe head trauma polygraphic studies have lead to interesting findings (2, 3, 4, 5, 13, 19). All these studies have been concerned with adult patients suffering from post-traumatic coma. To our knowledge no similar investigations of infants and young children or of patients with milder injuries have been undertaken.

Since every tenth child will at one time or another suffer from an injury to the head causing—usually mild—neurological symptoms (18) patients with head trauma are frequently encountered in pediatric hospitals and outpatient departments. In these patients the routine EEG when evaluated visual inspection alone is of limited value during the acute state (6, 10, 11, 15). It may even be quite inconspicuous a few hours after the accident in spite of clinical symptoms indicating functional or morphological changes in the brain or of a fracture proving the severity of the impact to the skull.

We were therefore interested in discovering whether polygraphic sleep recordings could be helpful in the investigation of infants and young children with head injuries and moreover whether they could provide new insight into the pathophysiology of traumatic disturbances in the brain.

### MATERIAL AND METHODS

Our material consisted of 15 patients aged 2 months to 3 1/2 years who had suffered a head injury. Polygraphic recordings of the first two cycles of normal night sleep (from the beginning of the first epoch of NREM sleep to the end of the second epoch of REM sleep) were made on two different occasions on each patient. The first recording was made within the first 5 days of the head injury and the second recording 1 to 3 weeks later. In this way each patient served as his own control and errors of interpretation due to interindividual variations in the duration of sleep stages and cycles and in the intensity of certain focal critical phenomena such as the sleep spindles could be avoided. Six other subjects from whom only one recording could be obtained have therefore not been included in this study.

Recordings were carried out with an 8 channel Offner type T polygraph with an ink write-out. Silver disc electrodes were fixed with gauze strips dipped into collodion at the following standard positions: F<sub>7</sub>, F<sub>8</sub>, C<sub>3</sub>, C<sub>4</sub>, D and O. The EEG was recorded bipolarly with a time constant of 0.5 sec and with an amplification of 50  $\mu$ V/5 mm at a paper speed of 15 mm/sec. Eye movements were recorded from electrodes fixed at the outer canthi of the left eye and at the bridge of the nose. Respiration and submental EMG were recorded simultaneously for additional monitoring of the sleep stages. All subjects were carefully ob-

For explanation see review article by H. G. Lenard in this issue.

- 5 Bloom G, Dunér H, Pernow B, Winberg J & Zetterstrom R Spontaneous histamine shocks in urticaria pigmentosa *Acta Paediatr* 47 152 1958
- 6 Brett E M, Ong H H & Friedmann T Mast cell disease in children *Brit J Derm* 79 197 1967
- 7 Brogren N, Dunér H, Hamrin B, Pernow B, Thander G & Waldenström J Urticaria pigmentosa (mastocytosis). A study of nine cases with special reference to the excretion of histamine in urine *Acta Med Scand* 163 223 1959
- 8 Burgeon C F Jr, Graham J H & McCaffree D L Mast cell disease *Arch Derm* 98 590 1968
- 9 Ciplan R M The natural course of urticaria pigmentosa *Arch Derm* 87 146 1963
- 10 Cole A R C Mastocytosis. Report of a case in a 15 1/2-week old infant with cutaneous and generalized skeletal changes *Arch Dis Child* 40 677 1965
- 11 Curry J J The action of histamine on the respiratory tract in normal and asthmatic subjects *J Clin Invest* 25 785 1946
- 12 Dagon R, Delort J, Verbae J & Civalle F Mastocytose a plaque unique du nourisson. Pousées bulleuses locales associées à une vaso-dilatation généralisée des téguments *Bull Soc Franc Derm Syph* 62 135 1955
- 13 Demis D J The mastocytosis syndrome. Clinical and biological studies *Ann Intern Med* 59 194 1963
- 14 Demis D J, Walton M D & Higdon R S Histaminuria in urticaria pigmentosa and the mastocytosis syndrome. A clinical study and review of the recent literature with definition of the mastocytosis syndrome *Arch Derm* 83 127 1961
- 15 Graciansky M P, Loewe-Lyon, Grupper C & Trieb R Mastocytose unique avec pousée bulleuse *Bull Soc Franc Derm Syph* 61 104 1954
- 16 Grunerus G Urinary excretion of histamine, methylhistamine and methylhistamizoleacetic acids in man under standardized dietary conditions *Scand J Clin Lab Invest* 22 Suppl 104 59 1968
- 17 Gross P Urticaria pigmentosa (solitary lesion) *Arch Derm* 29 451 1934
- 18 Johannsen L & Kunzer W Zum Verhalten der Blutgerinnung bei Urticaria Pigmentosa *Arch Kinderheilk* 174 133 1966
- 19 Johnson W & Helwig E Solitary mastocytosis *Arch Derm* 84 806 1961
- 20 Klaus S N & Winkelmann R K Course of urticaria pigmentosa in children *Arch Derm* 86 68 1962
- 21 Lindell S E, Rossmann H & Westling H Histamine formation in urticaria pigmentosa *Acta Dermatovenereol* 41 277 1961
- 22 Lynch F Urticaria pigmentosa (formerly bullous) accompanied by transient urticaria *Arch Derm* 71 668 1955
- 23 Marshall J, Walker J, Lurie H I, Hansen J D L & Mackenzie D Solitary mastocytoma and the mastocytoses: a discussion of the mastocytoses and a report of two cases of solitary mastocytoma showing an unusual phenomenon of generalized flushing *S Afr Med J* 31 867 1957
- 24 McKee W D, Cochrane C G & Farr R S A clinical study of an unusual case of asthma associated with urticaria pigmentosa *J Allerg* 37 38 1966
- 25 Morgan H, Gross B & Reissner R Solitary urticaria pigmentosa with systemic symptoms *Arch Derm* 88 473 1963
- 26 Mutter R H, Tannenbaum M & Uhlmann J E Systemic mast cell disease *Ann Intern Med* 59 887 1963
- 27 Nettleship E Rare forms of urticaria *Brit med J* 18 323 1869
- 28 Nilsson I M & Ölow B Determination of fibrinogen and fibrinolytic activity *Thromb Diath Haemorrhag* 8 297 1962
- 29 Nilsson I M & Wenckert A Demonstration of a heparin-like anticoagulant in normal blood *Acta Med Scand Suppl* 298 1954
- 30 Orkin M Mastocytosis in animals *Arch Derm* 96 381 1967
- 31 Öwren P A & Års K The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin *Scand J Clin Lab Invest* 3 201 1951
- 32 Pandolfi M, Isacson S, Nilsson I M & Robertson H Fibrinolytic activity of human veins *Lancet* 2 127 1967
- 33 Robinson H M, Hale R L, Hitch J M, Baileigh N C & Robinson R C V Bullous urticaria pigmentosa *Arch Derm* 85 346 1967
- 34 Sagher F & Even Piz Z *Mastocytosis and the mast cell* Karger, Basel and New York 1967
- 35 Shaw J M Genetic aspects of urticaria pigmentosa *Arch Derm* 97 137 1968
- 36 Westling H Evaluation of histamine metabolism in man *Scand J Clin Lab Invest* 1 23 1969
- 37 Wetterqvist H & White T Urinary excretion of histamine and methylhistamine in male and female rats *Scand J Clin Lab Invest* 22 Suppl 104 3 1968
- 38 Wolf P A modification for routine laboratory use of Stefanini's method of estimating factor V activity in human oxalated plasma *J Clin Path* 6 34 1953

Submitted Dec 12 1969

Accepted Jan 13 1970

Dept of Paediatrics  
Malmö Allmänna Sjukhus  
214 01 Malmö  
Sweden

Key words Mastocytoma infants histaminuria

## ALTERATIONS IN THE SLEEP PATTERNS OF INFANTS AND YOUNG CHILDREN FOLLOWING ACUTE HEAD INJURIES

II Q LENARD and H PENNINGSTORFF

*From the Department of Paediatrics University of Göttingen Göttingen West Germany*

Whereas sleep—frequently drug induced—is widely used in clinical electroencephalography as a method of provoking paroxysmal activity in certain epileptic patients the continuous recording by polygraphic techniques of several hours of normal night sleep is a new tool for the investigation of variety of pathologic conditions. Both qualitative and quantitative changes in the different stages of sleep may be shown thus revealing alterations in the structures responsible for the cyclic changes of brain functions.

In patients with severe head trauma polygraphic studies have lead to interesting findings (2, 3, 4, 5, 13, 19). All these studies have been concerned with adult patients suffering from post traumatic coma. To our knowledge no similar investigations of infants and young children or of patients with milder injuries have been undertaken.

Since every tenth child will at one time or another suffer from an injury to the head causing—usually mild—neurological symptoms (18) patients with head trauma are frequently encountered in pediatric hospitals and outpatient departments. In these patients the routine EEG when evaluated visual inspection alone is of limited value during the acute stage (6, 10, 11, 15). It may even be quite inconspicuous a few hours after the accident in spite of clinical symptoms indicating functional or morphological changes in the brain or of a fracture proving the severity of the impact to the skull.

We were therefore interested in discovering whether polygraphic sleep recordings could be helpful in the investigation of infants and young children with head injuries and moreover whether they could provide new insight into the pathophysiology of traumatic disturbances in the brain.

### MATERIAL AND METHODS

Our material consisted of 15 patients aged 2 months to 3 1/2 years who had suffered a head injury. Polygraphic recordings of the first two cycles of normal night sleep (from the beginning of the first epoch of NREM sleep to the end of the second epoch of REM sleep) were made on two different occasions on each patient. The first recording was made within the first 5 days of the head injury and the second recording 1 to 3 weeks later. In this way each patient served as his own control and errors of interpretation due to interindividual variations in the duration of sleep stages and cycles and in the intensity of certain basic focal phenomena such as the sleep spindles could be avoided. Six other subjects from whom only one recording could be obtained have therefore not been included in this study.

Recordings were carried out with an 8 channel Offner type T polygraph with an ink write-out. Silver disc electrodes were fixed with gauze strips dipped into collodion at the following standard positions: F<sub>7</sub>, F<sub>8</sub>, C<sub>3</sub>, C<sub>4</sub>, O and O'. The EEG was recorded bipolarly with a time constant of 0.3 sec and with an amplification of 50  $\mu$ V/5 mm at a paper speed of 15 mm/sec. Eye movements were recorded from electrodes fixed at the outer canthus of the left eye and at the bridge of the nose. Respiration and submental FMC were recorded simultaneously for additional monitoring of the sleep stages. All subjects were carefully ob-

For explanation see review article by H. Q. Lenard in this issue.



- 5 Bloom G, Dunér H, Pernow B, Winberg J & Zetterstrom R. Spontaneous histamine shocks in urticaria pigmentosa. *Acta Paediatr* 47 152 1958
- 6 Brett E M, Ong B H & Friedmann T. Mast cell disease in children. *Brit J Derm* 79 197 1967
- 7 Brogren N, Dunér H, Hamrin B, Pernow B, Theander G & Wildenstrom J. Urticaria pigmentosa (mastocytosis). A study of nine cases with special reference to the excretion of histamine in urine. *Acta Med Scand* 163 223 1959
- 8 Burgoon C F Jr, Graham J H & McCaffrey D L. Mast cell disease. *Arch Derm* 98 590 1968
- 9 Caplan R M. The natural course of urticaria pigmentosa. *Arch Derm* 87 146 1963
- 10 Cole A R C. Mastocytosis. Report of a case in a 15-week-old infant with cutaneous and generalized skeletal changes. *Arch Dis Child* 40 677 1965
- 11 Curry J J. The action of histamine on the respiratory tract in normal and asthmatic subjects. *J Clin Invest* 25 785 1946
- 12 Dugas H, Delort J, Verhuc F & Civatte F. Mastocytose à plaque unique du nourisson. Poussee bulleuse locale associee a une vasodilatation generalisee des teguments. *Bull Soc Franc Derm Syph* 62 135 1955
- 13 Demis D J. The mastocytosis syndrome. Clinical and biological studies. *Ann Intern Med* 59 194 1963
- 14 Demis D J, Walton M D & Higdon R S. Histaminuria in urticaria pigmentosa and the mastocytosis syndrome. A clinical study and review of the recent literature with definition of the mastocytosis syndrome. *Arch Derm* 83 127 1961
- 15 Gricansky M P, Loewe-Lyon Grupper C & Tueb R. Mastocytome unique avec pousse bulleuse. *Bull Soc Franc Derm Syph* 61 164 1954
- 16 Grinerus G. Urinary excretion of histamine, methylhistamine and methylimidazoleacetic acid in man under standardized dietary conditions. *Scand J Clin Lab Invest* 22 Suppl 104 39 1968
- 17 Gross P. Urticaria pigmentosa (solitary lesion). *Arch Derm* 29 451 1934
- 18 Johannsen L & Kunzer W. Zum Verhalten der Blutgerinnung bei Urticaria Pigmentosa. *Arch Kinderheilk* 174 133 1966
- 19 Johnson W & Helwig E. Solitary mastocytosis. *Arch Derm* 84 806 1961
- 20 Klaus S N & Winkelmann R K. Course of urticaria pigmentosa in children. *Arch Derm* 86 68 1962
- 21 Ludell S E, Roraman H & Westling H. Histamine formation in urticaria pigmentosa. *Acta Dermatovenereol* 41 277 1961
- 22 Lynch F. Urticaria pigmentosa (formerly bullous accompanied by transient urticaria). *Arch Derm* 71 668 1955
- 23 Marshall J, Walker J, Lurie H I, Hansen J D L & Mackenzie D. Solitary mastocytoma and the mastocytes: a discussion of the mastocytoses and a report of two cases of solitary mastocytoma showing an unusual phenomenon of generalized flushing. *S Afr Med J* 31 867 1957
- 24 McKee, W D, Cochrane C G & Farr R S. A clinical study of an unusual case of asthma associated with urticaria pigmentosa. *J Allergy* 37 38 1966
- 25 Morgan H, Gross B & Reissner R. Solitary urticaria pigmentosa with systemic symptoms. *Arch Derm* 88 473 1963
- 26 Mutter R D, Tannenbaum M & Ullmann J E. Systemic mast cell disease. *Ann Intern Med* 59 887 1963
- 27 Nettleship E. Rare forms of urticaria. *Brit med J* 18 323 1869
- 28 Nilsson I M & Olow B. Determination of fibrinogen and fibrinolytic activity. *Thromb Diath Haemorrhag* 8 297 1962
- 29 Nilsson I M & Wenckert A. Demonstration of a heparin-like anticoagulant in normal blood. *Acta Med Scand Suppl* 298 1954
- 30 Orkin M. Mastocytosis in animals. *Arch Derm* 96 381 1967
- 31 Owren P A & Aas K. The control of disseminated intravascular coagulation and the quantitative determination of prothrombin and proconvertin. *Scand J Clin Lab Invest* 5 201 1951
- 32 Pandolfi M, Isacson S, Nilsson I M & Robertson B. Fibrinolytic activity of human veins. *Lancet* 2 127 1967
- 33 Robinson H M, Kile R L, Hitch J M, Raleigh N C & Robinson R C V. Bullous urticaria pigmentosa. *Arch Derm* 85 346 1967
- 34 Sagher P & Even Paz Z. Mastocytosis and the mast cell. Harper & Row, New York 1967
- 35 Shaw J M. Genetic aspects of urticaria pigmentosa. *Arch Derm* 97 137 1968
- 36 Westling H. Evaluation of histamine metabolism in man. *Scand J Clin Lab Invest* 1 23 1969
- 37 Wetterqvist H & White T. Urinary excretion of histamine and methylhistamine in male and female rats. *Scand J Clin Lab Invest* 22 Suppl 104 3 1968
- 38 Wolf P. A modification for routine laboratory use of Stefanini's method of estimating factor V activity in human oxalated plasma. *J Clin Path* 6 34 1953

Submitted Dec 12 1969

Accepted Jan 13 1970

Dept of Paediatrics  
Malmö Allmänna Sjukhus  
214 01 Malmö  
Sweden

Key words: Mastocytoma; infants; histaminuria

## ALTERATIONS IN THE SLEEP PATTERNS OF INFANTS AND YOUNG CHILDREN FOLLOWING ACUTE HEAD INJURIES

H G LENARD and H PENNIGSTORFF

*From the Department of Paediatrics University of Göttingen Göttingen West Germany*

Whereas sleep—frequently drug induced—is widely used in clinical electroencephalography as a method of provoking paroxysmal activity in certain epileptic patients the continuous recording by polygraphic techniques of several hours of normal night sleep is a new tool for the investigation of variety of pathologic conditions. Both qualitative and quantitative changes in the different stages of sleep may be shown thus revealing alterations in the structures responsible for the cyclic changes of brain functions.

In patients with severe head trauma polygraphic studies have lead to interesting findings (2, 3, 4, 5, 13, 19). All these studies have been concerned with adult patients suffering from post-traumatic coma. To our knowledge no similar investigations of infants and young children or of patients with milder injuries have been undertaken.

Since every tenth child will at one time or another suffer from an injury to the head causing—usually mild—neurological symptoms (18) patients with head trauma are frequently encountered in pediatric hospitals and outpatient departments. In these patients the routine EEG when evaluated visual inspection alone is of limited value during the acute state (6, 10, 11, 15). It may even be quite inconspicuous a few hours after the accident in spite of clinical symptoms indicating functional or morphological changes in the brain or of a fracture proving the severity of the impact to the skull.

We were therefore interested in discovering whether polygraphic sleep recordings could be helpful in the investigation of infants and young children with head injuries and moreover whether they could provide new insight into the pathophysiology of traumatic disturbances in the brain.

### MATERIAL AND METHODS

Our material consisted of 15 patients aged 2 months to 3 1/2 years who had suffered a head injury. Polygraphic recordings of the first two cycles of normal night sleep (from the beginning of the first epoch of REM sleep to the end of the second epoch of REM sleep) were made on two different occasions on each patient. The first recording was made within the first 5 days of the head injury and the second recording 1 to 3 weeks later. In this way each patient served as his own control and errors of interpretation due to interindividual variations in the duration of sleep stages and cycles and in the intensity of certain bioelectrical phenomena such as the sleep spindles could be avoided. Six other subjects from whom only one recording could be obtained have therefore not been included in this study.

Recordings were carried out with an 8 channel Offner type T polygraph with an ink write-out. Silver disc electrodes were fixed with gauze strips dipped into collodion at the following standard positions: F<sub>4</sub>, F<sub>3</sub>, C<sub>4</sub>, C<sub>3</sub>, O and O. The EEG was recorded bipolarly with a time constant of 0.3 sec and with an amplification of 50  $\mu$ V/5 mm at a paper speed of 15 mm/sec. Eye movements were recorded from electrodes fixed at the outer canthus of the left eye and at the bridge of the nose. Respiration and submental EMG were recorded simultaneously for additional monitoring of the sleep stages. All subjects were carefully ob-

For explanation see review article by H G Lenard in this issue.

- 5 Bloom G Diner H Pernow B Winberg J & Zetterstrom R Spontaneous histamine shocks in urticaria pigmentosa *Acta Paediat* 47 152 1958
- 6 Brett E M Ong B H & Friedmann T Mast cell disease in children *Brit J Derm* 79 197 1967
- 7 Brogren N Diner H Hamrin B Pernow B Theander G & Waldenstrom J Urticaria pigmentosa (mastocytosis) A study of nine cases with special reference to the excretion of histamine in urine *Acta Med Scand* 163 223 1959
- 8 Burgoon C F Jr Graham J H & McCaffree D L Mast cell disease *Arch Derm* 98 590 1968
- 9 Caplin R M The natural course of urticaria pigmentosa *Arch Derm* 87 146 1963
- 10 Cole A R C Mastocytosis Report of a case in a 15 / week old infant with cutaneous and generalized skeletal changes *Arch Dis Child* 40 677 1965
- 11 Curry J J The action of histamine on the respiratory tract in normal and asthmatic subjects *J Clin Invest* 23 785 1946
- 12 Degos R Delort J Verhae F & Civatte F Mastocytose: une plaque unique du nourrisson. Poursuivie bulveuses locales associees a une vasodilatation generalisee des teguments *Bull Soc Franc Derm Syph* 62 135 1955
- 13 Demis D J The mastocytosis syndrome Clinical and biological studies *Ann Intern Med* 59 194 1963
- 14 Demis D J Walton M D & Higdon R S Histaminuria in urticaria pigmentosa and the mastocytosis syndrome A clinical study and review of the recent literature with definition of the mastocytosis syndrome *Arch Derm* 83 127 1961
- 15 Grunewald M P Loewe Lyon Grupper C & Tueb R Mastocytose unique avec poussée bulveuse *Bull Soc Franc Derm Syph* 61 104 1954
- 16 Grunewald G Urinary excretion of histamine methylhistamine and methylimidazoleacetic acids in man under standardized dietary conditions *Scand J Clin Lab Invest* 22 Suppl 104 59 1968
- 17 Gross F Urticaria pigmentosa (solitary lesion) *Arch Derm* 29 451 1934
- 18 Johannsen L & Kunzer W Zum Verhalten der Blutgerinnung bei Urticaria Pigmentosa *Arch Kinderheilk* 174 133 1966
- 19 Johnson W & Helwig E Solitary mastocytosis *Arch Derm* 84 806 1961
- 20 Khos S N & Winkelmann R K Course of urticaria pigmentosa in children *Arch Derm* 86 68 1962
- 21 Lindell S E Rorsman H & Westling H Histamine formation in urticaria pigmentosa *Acta Dermatoven* 41 277 1961
- 22 Lynch F Urticaria pigmentosa (formerly bullous) accompanied by transient urticaria *Arch Derm* 71 668 1955
- 23 Marshall J Walker J Lurie H I Hansen J D L & Mackenzie D Solitary mastocytosis and the mastocytosis a discussion of the mastocytosis and a report of two cases of solitary mastocytoma showing an unusual phenomenon of generalized flushing *S Afr Med J* 31 867 1957
- 24 McKeay W D Cochrane C G & Farr R S A clinical study of an unusual case of asthma associated with urticaria pigmentosa *J Allergy* 37 38 1966
- 25 Morgan H Gross H & Reissner R Solitary urticaria pigmentosa with systemic symptoms *Arch Derm* 83 473 1963
- 26 Mutter R D Tanenbaum M & Utman J E Systemic mast cell disease *Ann Intern Med* 59 887 1963
- 27 Nettleship E Rare forms of urticaria *Brit med J* 18 323 1869
- 28 Nilsson I M & Olow B Determination of fibrinogen and fibrinolytic activity *Thromb Diath Haemorrhag* 8 297 1962
- 29 Nilsson I M & Wenckert A Demonstration of a heparin like anticoagulant in normal blood *Acta Med Scand Suppl* 293 1954
- 30 Orkin M Mastocytosis in animals *Arch Derm* 96 381 1967
- 31 Owren P A & Aas K The control of thrombolytic therapy and the quantitative determination of prothrombin and proconvertin *Scand J Clin Lab Invest* 3 201 1951
- 32 Pandolfi M Isaacson S Nilsson I M & Robertson B Fibrinolytic activity of human veins *Lancet* 2 127 1967
- 33 Robinson H M Kile R L Hitch J M & Leigh N C & Robinson E C V Bullous urticaria pigmentosa *Arch Derm* 85 346 1967
- 34 Sagher F & Even Paz Z *Mastocytosis and the mast cell* Karger Basel and New York 1967
- 35 Shaw J M Genetic aspects of urticaria pigmentosa *Arch Derm* 97 137 1968
- 36 Westling H Evaluation of histamine metabolism in man *Scand J Clin Lab Invest* 1 23 1969
- 37 Wetterqvist H & White T Urinary excretion of histamine and methylhistamine in male and female rats *Scand J Clin Lab Invest* 22 Suppl 104 3 1968
- 38 Wolf P A modification for routine laboratory use of Steigman's method of estimating factor V activity in human ovalated plasma *J Clin Path* 6 34 1953

Submitted Dec 12 1969

Accepted Jan 13 1970

Dept of Paediatrics  
Malmö Allmänna Sjukhus  
214 01 Malmö  
Sweden

Key words Mastocytoma infants histaminuria

Table 1 Age of the patients and time of the two recordings. For every record percentage of REM sleep time and the values for the ratio stage 2/stage 3+4 and stage 2+3/stage 4 are given. The last two columns give the clinical diagnosis and the findings of the routine EEG

Subjects	Days after injury	Percentage of REM sleep	Stage 2	Stage 2+3	Diagnosis	Routine EEG findings
			stage 3+4	stage 4		
L.D.	2	30	0.09	5.05	Fracture of skull concussion	Right occ. slowing
2 mo	10	43	0.07	11.80		
M.K.	3	42	0.19	23.50	Fracture of skull	Right par-occ. depression
6 mo	11	36	0.11	11.70		
O.B.	2	13	1.75	36.00	Depressed orbital fracture operated on 4th day	Right par-occ. slowing
6 mo	10	17	0.69	30.14	Fracture of skull concus- sion chronic brain damage	General depression of amplitude already before injury
12 R	0	26				
9 mo	7	18				
A.N.	4	30	0.09	10.70	Fracture of skull	Normal
9 mo	12	20	0.02	7.70		
C.Z.	1	11	0.53	12.22	Concussion	Normal
10 mo	22	2	0.22	8.09		
A.S.	4	7	0.48	6.70	Depressed right par fracture	Normal
1 y	13	21	0.43	6.10		
A.W.	4	16	1.71	11.96	Fracture of skull	Normal
1 y	9	9	0.53	8.63		
U.B.	1	23	0.48	4.15	Fracture of skull	Normal
1 y	10	24	0.14	3.10		
M.F.	0	14	2.60	34.00	Concussion	Normal
1 y	8	27	1.14	5.22		
R.Z.	2	17	2.27	7.00	Concussion	Normal
2 y	16	11	0.69	2.60		
A.S.	1	13	1.21	39.10	Fracture of skull concussion	Normal
2 y	11	13	0.21	9.30		
M.S.	1	7	0.91	4.16	Concussion transient aphasia + paresis right arm	Normal
2 y	11	5	0.31	1.06		
P.S.	3	23	0.67	3.36	Concussion	Dysrhythmic
2 y	13	20	0.39	1.80		
M.W.	2	22	1.40	00	Concussion severe with mouth movements and gaze to left side	Dysrhythmic
3 y	9	31	0.50	5.40		

ings taken soon after the accident. In order to investigate whether there were additional changes in the amount of spindles per time unit and in the duration of spindles interval and duration histograms of the spindles during two epochs of NREM sleep were made. From these histograms (an example is given in Fig. 2) the median was computed the median being preferred to the mean value because of the marked asymmetry of some distributions. This rather cumbersome procedure was carried out in the first 10 patients studied and the results are given in Table 2. Since no relation has been found between the side of clinical symptoms and the median values for spindle intervals or spindle duration of one hemisphere the mean

value for both hemispheres has been used for statistical computation. The intervals were shorter during the acute stage after the injury in all 10 patients. Less consistent changes were observed in the duration of the spindles. Seven out of ten patients showed an increase in spindle duration after the injury while in 3 patients a decrease was found. When there was an increase however the median value was considerably higher in the first record when compared with the second while in those subjects who had shorter spindles in the first record only a slight decrease was found in the second. Using Wilcoxon's matched-pairs signed-ranks test  $p$  was found to be less than 0.05 and greater than 0.02 in two-tailed test.

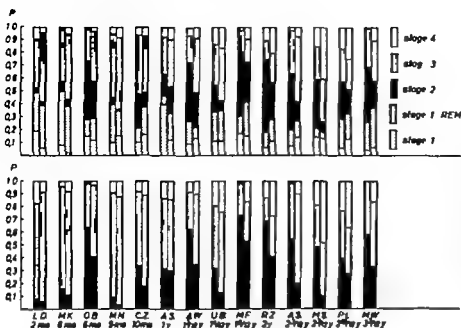


Fig 1 Upper row: Probability density for the different sleep stages during the acute stage after the injury (left column) and during the control record 13 weeks later (right column). Lower row: Probability density for the different stages of NREM sleep. Note increase of stage 2 and reduction of stage 4 in the record following the trauma.

served during the recording and behavioural patterns such as gross movements, mouthing and vocalization were noted on the record.

For every page of the recording (20 sec) the sleep stage was classified according to the criteria of Dement & Kleitman (7). Only 20 sec periods with at least one eye movement were classified as REM sleep. The number of eye movements per minute during REM sleep was counted from the electro-oculographic recording. In the leads  $F_4-C_2$  and  $F_3-C_2$  of the EEG the duration of the sleep spindles and the duration of the intervals between the beginning of one spindle and the beginning of the next were measured with a ruler. A spindle was defined as a regular sequence of 11–14 cps waves of at least 4 mm (4/15 sec) duration. Intervals longer than 100 sec and those during which a gross body movement occurred were not counted.

## RESULTS

### 1 Quantitative changes of sleep cycles

In one patient (UR) a child with severe chronic brain damage the EEG showed low voltage and high frequency continuously during NREM sleep in both recordings and it was not possible to discriminate the different stages. In the remaining 14 patients an increase of stage 2 (light sleep) and a relative decrease of the stage 3 and 4 (intermediate and deep sleep) were shown during NREM sleep in the first recording made after the injury. During this acute stage 13 patients had a higher percentage of the NREM stages with spindles (stages 2 and

3) and a lower percentage of stage 4 which is characterized by high amplitude slow activity and an absence of spindles. Only in the youngest patient aged 2 months initially was the opposite pattern found i.e. more stage 4 during the first recording. Since sleep spindles only appear in the EEG between the second and third month of life the increase of spindle stages in the second recording in this patient may have been due to the fact that the normal developmental changes exceeded the changes caused by the trauma.

No consistent changes of total REM sleep time have been found. In 9 patients a higher percentage of REM sleep was found in the first record and in 6 patients more REM sleep was found during the second record.

Fig 1 shows the probability density of the different sleep stages for the two recordings in 14 patients. In Table 1 the percentages of REM sleep time and the values for the ratio stage 2/stages 3+4 and stages 2+3/stage 4 are shown together with the clinical findings in all subjects.

### 2 Changes of spindle activity

As mentioned earlier, an increase of NREM sleep stages with spindles and a relative decrease of stage 4 was found in the first record

Table 1 Age of the patients and time of the two recordings. For every record percentage of REM sleep time and the values for the ratio stage 2/stage 3+4 and stage 2+3/stage 4 are given. The last two columns give the clinical diagnosis and the findings of the routine EEG

Subjects age	Days after injury	Percentage of REM sleep	Stage 2	Stage 2+3	Diagnosis	Routine EEG findings
			stage 3+4	stage 4		
L. D.	2	30	0.09	5.05	Fracture of skull contusion	Right occ. slowing
2 mo	10	45	0.07	11.80	Fracture of skull	Right par.-occ. depression
M. R.	3	42	0.19	23.90		
6 mo	11	36	0.11	11.70		
O. B.	2	13	1.73	36.00	Depressed orbital fracture operated on 3rd day	Right par.-occ. slowing
6 mo	10	17	0.69	30.14		
U. R.	0	26			Fracture of skull concus- sion chronic brain damage	Cerebral depression of amplitude already before injury
9 mo	7	18				
M. N.	4	30	0.09	10.70	Fracture of skull	Normal
9 mo	12	20	0.02	7.70		
C. Z.	1	11	0.33	12.22	Concussion	Normal
10 mo	22	22	0.22	8.09		
A. S.	4	7	0.48	6.70	Depressed right par. fracture	Normal
1 y	13	21	0.43	6.10		
A. W.	4	18	1.71	11.96	Fracture of skull	Normal
1 y	9	9	0.53	8.63		
U. B.	1	23	0.48	4.15	Fracture of skull	Normal
1 y	10	24	0.14	3.10		
M. F.	0	14	2.60	34.00	Concussion	Normal
1 y	8	27	1.14	5.22		
R. Z.	2	17	2.27	7.00	Concussion	Normal
2 y	18	31	0.69	2.60		
A. S.	1	13	1.21	39.10	Fracture of skull contusion	Normal
2 y	11	13	0.21	9.50		
K. S.	1	7	0.91	4.16	Contusion transient aphasia + paresis right arm	Normal
2 y	11	3	0.11	1.06		
P. S.	5	23	0.67	3.36	Concussion	Dysrhythmic
2 y	13	0	0.39	1.80		
M. W.	2	22	1.46	∞	Contusion fracture with mouth movements and gaze to left side	Dysrhythmic
3 y	9	31	0.30	5.40		

ings taken soon after the accident. In order to investigate whether there were additional changes in the amount of spindles per time unit and in the duration of spindles interval and duration histograms of the spindles during two epochs of NREM sleep were made. From these histograms (an example is given in Fig. 2) the median was computed, the median being preferred to the mean value because of the marked asymmetry of some distributions. This rather cumbersome procedure was carried out in the first 10 patients studied and the results are given in Table 2. Since no relation has been found between the side of clinical symptoms and the median values for spindle intervals or spindle duration of one hemisphere the mean

value for both hemispheres has been used for statistical computation. The intervals were shorter during the acute stage after the injury in all 10 patients. Less consistent changes were observed in the duration of the spindles. Seven out of ten patients showed an increase in spindle duration after the injury while in 3 patients a decrease was found. When there was an increase however the median value was considerably higher in the first record when compared with the second while in those subjects who had shorter spindles in the first record only a slight decrease was found in the second. Using Wilcoxon's matched-pairs signed-ranks test  $p$  was found to be less than 0.05 and greater than 0.02 in two-tailed test.

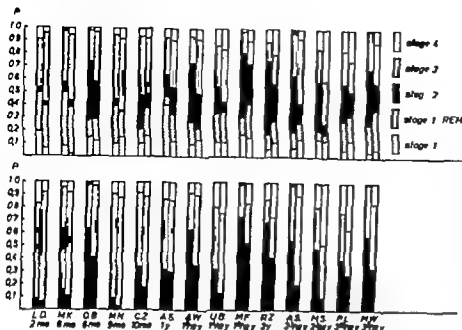


Fig 1 Upper row Probability density for the different sleep stages during the acute stage after the injury (left column) and during the control record 1-3 weeks later (right column) Lower row Probability density for the different stages of NREM sleep Note increase of stage 2 and reduction of stage 4 in the record following the trauma

served during the recording and behavioural patterns such as gross movements, mouthing and vocalization were noted on the record.

For every page of the recording (20 sec) the sleep stage was classified according to the criteria of Dement & Kleitman (7). Only 20 sec periods with at least one eye movement were classified as REM sleep. The number of eye movements per minute during REM sleep was counted from the electro-oculographic recording. In the leads  $F_7-C_3$  and  $F_8-C_4$  of the EEG the duration of the sleep spindles and the duration of the intervals between the beginning of one spindle and the beginning of the next were measured with a ruler. A spindle was defined as a regular sequence of 11-14 cps wave of at least 4 mm (4,15 sec) duration. Intervals longer than 100 sec and those during which a gross body movement occurred were not counted.

## RESULTS

### 1 Quantitative changes of sleep cycles

In one patient (U R) a child with severe chronic brain damage the EEG showed low voltage and high frequency continuously during NREM sleep in both recordings and it was not possible to discriminate the different stages. In the remaining 14 patients an increase of stage 2 (light sleep) and a relative decrease of the stage 3 and 4 (intermediate and deep sleep) were shown during NREM sleep in the first recording made after the injury. During this acute stage 13 patients had a higher percentage of the NREM stages with spindles (stages 2 and

3) and a lower percentage of stage 4 which is characterized by high amplitude, slow activity and an absence of spindles. Only in the youngest patient aged 2 months initially was the opposite pattern found i.e. more stage 4 during the first recording. Since sleep spindles only appear in the EEG between the second and third month of life the increase of spindle stages in the second recording in this patient may have been due to the fact that the normal developmental changes exceeded the changes caused by the trauma.

No consistent changes of total REM sleep time have been found. In 11 patients a higher percentage of REM sleep was found in the first record and in 6 patients more REM sleep was found during the second record.

Fig 1 shows the probability density of the different sleep stages for the two recordings in 14 patients. In Table 1 the percentages of REM sleep time and the values for the ratio stage 2/stages 3+4 and stages 2+3/stage 4 are shown together with the clinical findings in all subjects.

### 2 Changes of spindle activity

As mentioned earlier an increase of NREM sleep stages with spindles and a relative decrease of stage 4 was found in the first record.

bral trauma the brain stem and in particular the mesencephalo-diencephalic structures represent the most vulnerable region where all lines of force must converge. Neurophysiological evidence for this assumption is provided by experiments with monkeys carried out by Ward (24) after concussion the activity recorded from the brain stem reticular formation showed far greater and longer depression than that recorded from cortical and other subcortical structures. Since the most important structures for the regulation of sleep cycles are located in the brain stem it was not surprising to find almost regularly that transient alterations of sleep patterns after head injuries occurred even in cases in which symptoms were only mild and no EEG alterations could be detected by routine visual analysis.

For clinical purposes these changes in sleep pattern are of limited value during the acute stage after the injury. The alterations are only slight and the inter- and intra-individual variability in the duration of sleep stages and cycles may be considerable (21, 23). Moreover a remarkably large number of children suffering from head injury are 'high risk' children coming from low social classes, presenting an anamnesis of previous trauma or of other cerebral disturbances (12). In these children one EEG after an accident is difficult to evaluate unless previous recordings are at hand. The need for a dynamic interpretation of the post-traumatic EEG stressed by Kellaway (14) and Rodin (20) exists also for the evaluation of polygraphic sleep recordings. Our findings show that a child in whom symptoms have disappeared and the EEG appears normal may not be recovered completely after a head injury. Functional disturbances in certain structures of the brain stem may still persist and we consider that a cautious approach in the management of these patients is warranted.

The results of our study—reduction of sleep stages with highly synchronized EEG activity, increase in amount and duration of sleep spindles and increase in the amount of rapid eye movements per time unit after acute head in-

jury—may give rise to some thoughts about the pathophysiology of head traumas. An increase of stage 2 sleep with reduction of the percentage of stage 3 and stage 4 sleep has been observed by Adey *et al* (1) in patients with chronic high cervical lesions. These authors suggest that the reason for these changes might be a decreased proprioceptive input. The same changes in the percentage of NREM stages have been seen by Bergamasco *et al* (2) in those patients with post-traumatic coma who showed sleep-like cyclic fluctuations of the EEG pattern. Interesting results have been obtained by Weitzman *et al* (25) in monkeys following the administration of *p*-chlorophenylamine, a selective depletor of serotonin which caused not only a reduction of total NREM time but increased values for the ratio stages 1+2 to stage 3+4 as well. Sachs (22) found that the cerebral spinal fluid (CSF) level of serotonin rose in both animals and man after concussion. This suggests that an increased flow of serotonin from the brain into the CSF may occur thus diminishing the content of serotonin in the serotonergic neurons in the raphe nuclei of the brain stem which would explain the relative increase in light sleep following head injuries.

In our patients we were unable to demonstrate a significant change of the percentage of REM sleep time as described by Adey *et al* (1) and by Bergamasco *et al* (3). The structures responsible for the organization of REM sleep in the nucleus reticularis pontis are perhaps less susceptible to traumatic lesions which could be of biological value regarding the importance of the REM sleep for the organism as shown in sleep deprivation experiments. The relatively mild trauma sustained in most of our patients may not have been sufficient to alter REM sleep to a degree detectable by our methods. We have however studied one child (which could not be included in our study because we were unable to obtain a second control recording) with neurological symptoms of brain stem damage who had no REM sleep during more than three hours of recording.



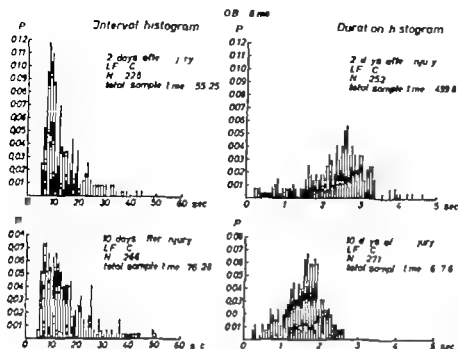


Fig 2 Example for interval and duration histograms of sleep spindles. The intervals between the spindles are shorter and the spindles are longer during the acute stage after the head trauma.

ing which suggests a strong tendency of the spindle length to increase after an acute head injury.

The frequency of the waves within the spindles was 13–14 cps in both recordings in all subjects.

### 3 Changes in the amount of rapid eye movements during REM sleep

Whereas the time spent in REM sleep and the percentage of REM sleep in total sleep time did not show consistent changes after the injury

the number of rapid eye movements was higher in 12 out of 14 patients (Table 3). In one patient (A SZ) the eye movements could not be counted because of a technical artefact in one record. The increase in the amount of eye movements is significant at the 1% level (Wilcoxon matched-pairs signed-ranks test two tailed).

## DISCUSSION

Denny Brown & Russel (8) concluded from animal experiments that in closed cranial-cere

Table 2 Median of length of the intervals between spindles (left column) and median of spindle duration (right column) during two epochs of NREM sleep in the record shortly after the trauma and in the control record

Subject	Spindle intervals sec		Spindle duration sec	
	1st record	2nd record	1st record	2nd record
M K	10.94	12.65	1.55	1.43
O B	11.35	13.45	2.52	1.57
U R	10.10	13.70	2.04	1.76
M N	11.75	13.15	1.56	1.47
C Z	11.10	11.50	1.45	1.49
A S	12.60	15.65	0.78	0.79
U B	14.50	28.55	1.22	0.90
M F	15.10	21.85	0.75	0.54
A S	14.55	18.45	1.06	0.94
M S	19.45	23.50	0.62	0.66

Table 3 Mean number of rapid eye movements per minute in the record following the trauma (left column) and the control record (right column)

Subject	1st record	2nd record
L D	17.0	14.8
M K	28.8	21.2
O B	12.9	7.9
U R	26.1	24.6
M N	27.1	10.1
C Z	10.1	6.6
A S	6.2	4.9
A W	16.5	9.9
U B	16.2	12.4
M F	15.6	19.2
R Z	10.2	8.6
M S	15.8	2.3
P L	23.5	8.3
M W	11.5	14.1

bral trauma the brain stem and in particular the mesencephalo-diencephalic structures represent the most vulnerable region where all lines of force must converge. Neurophysiological evidence for this assumption is provided by experiments with monkeys carried out by Ward (24) after concussion the activity recorded from the brain stem reticular formation showed far greater and longer depression than that recorded from cortical and other subcortical structures. Since the most important structures for the regulation of sleep cycles are located in the brain stem it was not surprising to find almost regularly that transient alterations of sleep patterns after head injuries occurred even in cases in which symptoms were only mild and no EEG alterations could be detected by routine visual analysis.

For clinical purposes these changes in sleep pattern are of limited value during the acute stage after the injury. The alterations are only slight and the inter and intrasubject variability in the duration of sleep stages and cycles may be considerable (21, 23). Moreover a remarkably large number of children suffering from head injury are "high risk" children coming from low social classes, presenting an anamnesis of previous trauma or of other cerebral disturbances (12). In these children one EEG after an accident is difficult to evaluate unless previous recordings are at hand. The need for a dynamic interpretation of the post-traumatic EEG stressed by Kellaway (14) and Rodin (20) exists also for the evaluation of polygraphic sleep recordings. Our findings show that a child in whom symptoms have disappeared and the EEG appears normal may not be recovered completely after a head injury. Functional disturbances in certain structures of the brain stem may still persist and we consider that a cautious approach in the management of these patients is warranted.

The results of our study—reduction of sleep stages with highly synchronized EEG activity, increase in amount and duration of sleep spindles and increase in the amount of rapid eye movements per time unit after acute head in-

jury—may give rise to some thoughts about the pathophysiology of head traumas. An increase of stage 2 sleep with reduction of the percentage of stage 3 and stage 4 sleep has been observed by Adey *et al.* (1) in patients with chronic high cervical lesions. These authors suggest that the reason for these changes might be a decreased proprioceptive input. The same changes in the percentage of NREM stages have been seen by Bergamasco *et al.* (2) in those patients with post-traumatic coma who showed sleep—like cyclic fluctuations of the EEG pattern. Interesting results have been obtained by Wertzman *et al.* (25) in monkeys following the administration of *p*-chlorophenylamine, a selective depletor of serotonin which caused not only a reduction of total NREM time but increased values for the ratio stages 1+2 to stage 3+4 as well. Secher (22) found that the cerebral spinal fluid (C.S.F.) level of serotonin rose in both animals and man after concussion. This suggests that an increased flow of serotonin from the brain into the C.S.F. may occur thus diminishing the content of serotonin in the serotonergic neurons in the raphe nuclei of the brain stem which would explain the relative increase in light sleep following head injuries.

In our patients we were unable to demonstrate significant changes of the percentage of REM sleep time as described by Adey *et al.* (1) and by Bergamasco *et al.* (3). The structures responsible for the organization of REM sleep in the nucleus reticularis pontis are perhaps less susceptible to traumatic lesions which could be of biological value regarding the importance of the REM sleep for the organism as shown in sleep deprivation experiments. The relatively mild trauma sustained in most of our patients may not have been sufficient to alter REM sleep to a degree detectable by our methods. We have however studied one child (which could not be included in our study because we were unable to obtain a second control recording) with neurological symptoms of brain stem damage who had no REM sleep during more than three hours of recording.

11-14 cps activity indistinguishable from sleep spindles has been seen in patients with post traumatic coma by Chatrian *et al* (5) Bergamasco *et al* (3) and Lucking (17) have described the gradual reappearance of sleep spindles in the EEG of patients recovering from profound posttraumatic coma. None of these authors offer an explanation for these findings. We also are unable to give more than a speculative explanation for the slight but consistent increase of spindle activity in our patients. Because of the statistical properties of spindles and REM in normal infants we have hypothesized that both phenomena might be the result of random noise in certain brain structures in the absence of patterning sensory inflow (16). Pre- and postsynaptic inhibition of various afferents during bursts of REM has been demonstrated in animal experiments whereas only indirect indications exist for the occurrence of such a mechanism during spindle discharges. Experimental data by Foltz & Schmidt (9) suggest a failure in conduction of peripheral afferent stimulation through the brain stem after concussion. If our hypothesis on the mechanism of spindles and REM holds true such an afferent inhibition could be the cause of the increase in spindles and REM after head injuries.

### SUMMARY

The first two cycles of normal night sleep were polygraphically recorded in 15 infants and young children during the acute stage after head injuries. Control records were carried out 1-3 weeks later.

Following the trauma there was an increase in stage 2 sleep and a relative reduction of the stages 3 and 4. During NREM sleep the percentage of stages with spindles (2 and 3) was higher than that of stage 4. No consistent changes in the percentage of REM sleep could be demonstrated.

The intervals between spindles were shorter after the trauma and in most cases the duration of spindles was longer. The amount of rapid eye movements per minute was higher during

the acute stage. The implications for the pathophysiology of head injuries in young infants are discussed.

### ACKNOWLEDGEMENTS

This investigation was supported by grants from the Deutsche Forschungsgemeinschaft, Sfb 120.

The technical assistance of Mrs Ulla Jurgens and the help of Dr W S Whurnup in preparing the manuscript are gratefully acknowledged.

### REFERENCES

- 1 Adey W R, Bors E & Porter R W. EEG sleep patterns after high cervical lesions in man. *Arch Neurol* 19: 377, 1968.
- 2 Bergamasco B, Bergamini L & Donguzzi T. Clinical value of the sleep electroencephalographic pattern in post traumatic coma. *Acta Neurol Scand* 44: 495, 1968.
- 3 Bergamasco B, Bergamini L, Donguzzi T & Fabiani D. EEG sleep patterns as a prognostic criterion in post traumatic coma. *Electroenceph Clin Neurophysiol* 24: 374, 1968.
- 4 Bergamasco B, Donguzzi T, Sacerdote I & Chusani C. Registrations poligraphiche notturne in soggetti in coma post traumatico. *Boll Soc Ital Biol Sper* 43: 322, 1966.
- 5 Chatrian G E, White L M & Daly D. Electroencephalographic patterns resembling those of sleep in certain comatose states after injuries to the head. *Electroenceph Clin Neurophysiol* 15: 272, 1963.
- 6 Crestard P, Chodkiewicz J P & Thebaud A. Etude à propos de cent cas de traumatisme crânio-cérébral chez le nourisson. *Neuro Chir (Paris)* 14: 759, 1968.
- 7 Dement W & Kleitman N. Cyclic variations in EEG during sleep and their relations to eye movements, body motility and dreaming. *Electroenceph Clin Neurophysiol* 9: 673, 1957.
- 8 Denny Brown D & Russell J. Experimental cerebral concussion. *Brain* 64: 93, 1941.
- 9 Foltz E L & Schmidt R H. The role of the reticular formation in the control of head injury. *J Neurosurg* 13: 145, 1956.
- 10 Frantzen E, Harvald B & Haugsted H. Fresh head injuries. *Acta Psych Scand* 33: 417, 1958.
- 11 Harris P. Head injuries in childhood. *Arch Dis Child* 32: 488, 1957.
- 12 Hjerrn H & Nylander I. Acute head injuries in children. *Acta Paediat Scand* 53: Suppl 152, 1964.
- 13 Jouvet M, Pellin B & Mounier D. Polygraphic study of the different sleep phases during chronic disturbances of consciousness (prolonged comas). *Electroenceph Clin Neurophysiol* 14: 138, 1962.

- 14 Kellaway P. Head injuries in children. *Electroenceph Clin Neurophysiol* 7: 497, 1955.
- 15 Lenard H. EEG Veränderungen bei frischen Schädeltraumen im Kindesalter. *Munch Med Woch* 107: 1820, 1965.
- 16 — The development of sleep spindles in the EEG during the first two years of life. *Neuropediatrics* 1: 264, 1970.
- 17 Luckert C. M. *Klinische und elektroencephalographische Befunde des Hirntodes nach schweren Hirnverletzungen*. Paper read at the annual meeting of the German EEG Society Bonn, 1969.
- 18 Melchior J. The incidence of head injuries in children. *Acta Paediatr Scand* 50: 47, 1961.
- 19 Pannouret P, Cadihac J, Delange M, Baldy Mouchier M & El Kasmaygi N. E. Différents stades électriques et organisation en cycle des comas post-traumatiques. Enregistrement polygraphique de longue durée. *Rev Neurol* 111: 391, 1964.
- 20 Rodin E. A. Contribution of the EEG to prognosis after head injury. *Dis Nerv Syst* 28: 595, 1967.
- 21 Roffwarg H. P., Muzio J. N. & Dement W. C. Ontogenetic development of the human sleep-wake cycle. *Science* 152: 604, 1966.
- 22 Sacha E. Acetylcholine and serotonin in the spinal fluid. *J Neurosurg* 14: 22, 1957.
- 23 Stern E, Parmelee A. H., Akiyama Y., Schultz, M. A. & Wenner W. H. Sleep cycle characteristics in infants. *Pediatrics* 43: 65, 1969.
- 24 Ward A. A. Physiological basis of concussion. *J Neurosurg* 15: 129, 1958.
- 25 Weissman E. D., Rapport M. M., McGrager P. & Jacoby J. Sleep patterns of the monkey and brain serotonin concentration: Effect of p-chlorophenylalanine. *Science* 160: 1361, 1968.

Submitted Dec. 1, 1969

Accepted Jan. 8, 1970

(H. G. L.) Dept. of Paediatrics  
University of Göttingen  
Humboldtallee 38  
Göttingen, West Germany

**Key words:** Head trauma, EEG, sleep.

11-14 cps activity indistinguishable from sleep spindles, has been seen in patients with post traumatic coma by Chatrian *et al* (5) Bergamasco *et al* (3) and Lucking (17) have described the gradual reappearance of sleep spindles in the EEG of patients recovering from profound posttraumatic coma. None of these authors offer an explanation for these findings. We also are unable to give more than a speculative explanation for the slight but consistent increase of spindle activity in our patients. Because of the statistical properties of spindles and REM in normal infants we have hypothesized that both phenomena might be the result of random noise in certain brain structures in the absence of patterning sensory inflow. (16) Pre and postsynaptic inhibition of various afferents during bursts of REM has been demonstrated in animal experiments whereas only indirect indications exist for the occurrence of such a mechanism during spindle discharges. Experimental data by Foltz & Schmidt (9) suggest a failure in conduction of peripheral afferent stimulation through the brain stem after concussion. If our hypothesis on the mechanism of spindles and REM holds true such an afferent inhibition could be the cause of the increase in spindles and REM after head injuries.

### SUMMARY

The first two cycles of normal night sleep were polygraphically recorded in 15 infants and young children during the acute stage after head injuries. Control records were carried out 1-3 weeks later.

Following the trauma there was an increase in stage 2 sleep and a relative reduction of the stages 3 and 4. During NREM sleep the percentage of stages with spindles (2 and 3) was higher than that of stage 4. No consistent changes in the percentage of REM sleep could be demonstrated.

The intervals between spindles were shorter after the trauma and in most cases the duration of spindles was longer. The amount of rapid eye movements per minute was higher during

the acute stage. The implications for the pathophysiology of head injuries in young infants are discussed.

### ACKNOWLEDGEMENTS

This investigation was supported by grants from the Deutsche Forschungsgemeinschaft, Sph 120.

The technical assistance of Mrs Ulla Jurgens and the help of Dr W S Whamish in preparing the manuscript are gratefully acknowledged.

### REFERENCES

- 1 Adey W R, Bors E & Porter R W. EEG sleep patterns after high cervical lesions in man. *Arch Neurol* 19: 377 1968.
- 2 Bergamasco B, Bergamini L & Donguzzi T. Clinical value of the sleep electroencephalographic pattern in post traumatic coma. *Acta Neurol Scand* 44: 495 1968.
- 3 Bergamasco B, Bergamini L, Donguzzi T & Fabiani D. EEG sleep patterns as a prognostic criterion in post traumatic coma. *Electroenceph Clin Neurophysiol* 24: 374 1969.
- 4 Bergamasco B, Donguzzi T, Stendero I & Chionso C. Registrazioni poligrafiche notturne in soggetti in coma post traumatico. *Boll Soc Ital Biol Sper* 43: 322 1966.
- 5 Chatrian G H, White L H & Daly D. Electroencephalographic patterns resembling those of sleep in certain comatose states after injuries to the head. *Electroenceph Clin Neurophysiol* 15: 272 1963.
- 6 Cressier P, Chodkiewicz J P & Thébaud A M. A propos de cent cas de traumatisme crânio-cérébral chez le nourisson. *Neuro Chir (Paris)* 14: 759 1969.
- 7 Dement W & Kleitman N. Cycle variations in EEG during sleep and their relations to eye movements, body motility and dreaming. *Electroenceph Clin Neurophysiol* 9: 673 1957.
- 8 Denny Brown D & Russell J. Experimental cerebral concussion. *Brain* 64: 93 1941.
- 9 Foltz E L & Schmidt R H. The role of the reticular formation in the coma of head injury. *J Neurosurg* 13: 145 1956.
- 10 Frantzen E, Hurvold B & Haugsted H. Fresh head injuries. *Acta Paed Scand* 33: 417 1954.
- 11 Harris P. Head injuries in childhood. *Arch Dis Child* 37: 489 1957.
- 12 Hjern B & Nylander I. Acute head injuries in children. *Acta Paediat Scand* 53: Suppl 152: 1 1964.
- 13 Jouvet M, Pellin B & Mounier D. Polygraphic study of the different sleep phases during chronic disturbances of consciousness (prolonged comas). *Electroenceph Clin Neurophysiol* 14: 138 1962.

sleep occurs in all mammalian species from man to the opossum

In sleeping human infants Zipferling (150) observed as early as 1913 repeatedly occurring periods of REM with irregular respiration and body motility but the significance of this observation was not understood until Kleitman & Engelman (77) and Aserinsky & Kleitman (6) reported the finding of an alternating sleep pattern also in the very young age group. Since then meticulous behavioural observations (10 148 149) and comprehensive polygraphic investigations (25 34 35 37 38 39 40 53 91 102 103 104 108 109 110 115 116 132) have been carried out on sleeping premature and full term newborns. Fewer reports have been published about the sleep pattern of infants and older children (47 79 128 141)

### NREM SLEEP

Synonyms for NREM sleep are regular sleep (148) quiet sleep (31 101) ruhiger Schlaf (66 119) sommeil calme (91) state 1 (114). In the neurophysiological literature one finds the terms synchronized sleep (68) high voltage slow wave sleep (26) telecephalic sleep (67). Both "deep sleep" and "light sleep" have been used for the description of this sleep state and it is obvious that these terms must be discarded in order to avoid misunderstandings.

During NREM sleep the infant is lying quiet except for irregularly occurring sudden generalised movements called startles. Startles are not identical with the Moro response; they occur without external stimulation and they do not consist of a regular pattern of muscular activity (114). After the newborn period startles become rare. Tonic activity can be recorded from the submental muscles; this phenomenon does not always occur especially in very young infants (41). Both respiratory rate and heart rate are slow and regular (116); the systolic blood pressure is low and stable (35). There are no movements of the eye balls after the newborn period; slow regular rolling movements

may occur at the onset of the first sleep cycle (27).

The EEG of NREM sleep undergoes marked developmental changes. Until 44–46 weeks conceptional age the dominating pattern is the so called trace alternant (131) consisting of longer periods with low voltage activity interrupted by periods of irregular high delta and theta waves 1–4 sec long. The flat periods which may show even a zero line EEG before 32 weeks gestational age increase in amplitude with increasing age. The bursts of the trace alternant are mixed with low amplitude beta activity at 32 weeks gestational age with sharp waves at 36 weeks and are made up of bilaterally synchronous delta and theta waves at term. During the second month after term the trace alternant is replaced by a sequence of EEG patterns corresponding to the NREM stages of adults classified by Dement & Kleitman (28): stage 1 any pattern between wakefulness and the appearance of 12–14 cps spindles; stage 2 low voltage EEG with spindles and occasional slow waves; stage 3 intermediate stage with high amplitude slow activity and spindles; stage 4 high amplitude delta and sub-delta waves without spindles. Changes in the relative amount of NREM stages during the first years of life are mainly due to a change in the amount of spindles. This peculiar EEG pattern appears during the second month of life; spindles are longest and most frequent during the third and fourth month thereafter they decrease in amount and length until they are restricted to stage 2 at the beginning of a NREM epoch (83). This development results in a continuous decrease of stage 3 and a relative increase of stage 4 while there is little change in the amount of stage 2.

In animals (7 52 81) human adults (57) and in human infants (58 117 120) proprioceptive reflexes undergo no or only little depression during NREM sleep as compared to wakefulness. This is also the case for the Moro reflex in infants. Exteroceptive reflexes and the vestibulo-ocular response (14 123) on the other hand are markedly depressed or disap-

## REVIEW ARTICLE

### SLEEP STUDIES IN INFANCY

#### *Facts Concepts and Significance*

H G LENARD

*From the Department of Paediatrics University of Göttingen Göttingen West Germany*

Although infants and young children spend the major part of their time sleeping pediatricians have for a long time shown surprisingly little interest in the physiology and pathology of sleep. Traditional concepts were handed over almost unchanged from one generation of pediatricians to the next. Sleep was simply regarded as the opposite of wakefulness as a period of recreation for the organism (trophotropic phase) in which all vegetative and neural systems were switched to a lower level of activity. The large amount of sleep required in the young though no longer contributed to anoxia of the infantile brain caused by excessive intake of milk (20-122) was explained by the greater fatigueability of the immature brain.

Immaturity and sleep are closely related. Those brain centers which just started functioning tend to relapse into the state they were in before being engaged into brain function (107). During the last 30 years however studies of theoretical and clinical neurophysiologists, psychologists, psychiatrists and a few pediatricians have led to such a wealth of new information that the 4377 references in Kleitman's famous book

Sleep and Wakefulness (76) are today no longer a complete bibliography. Recent research has shown the possibilities of applied sleep research especially in young infants. It therefore seems justified to review the results of recent sleep research to line out the present concept of

sleep and to discuss their possible relevance for the pediatrician, the pediatric neurologist and the student of human development.

#### THE DUALISTIC NATURE OF SLEEP

When the pioneers of electroencephalography found different patterns of bioelectric brain activity during sleep (86) they attributed this finding to different levels of sleep depth. In 1953 Aserinsky & Kleitman (4) found that a peculiar EEG pattern resembling that of wakefulness occurred periodically during sleep and coincided with rapid eye movements (REM) under the closed lids. During the epochs with REM the subjects showed various forms of motor activity (28-30), heart rate and respiratory rate increased (3) and the tonic activity of the anti-gravity muscles disappeared (11-63). The REM epochs were found to be closely related to the subjective experience of dreaming (5-29). These discoveries together with the results of neurophysiological studies—especially of Jouvet's group at Lyon and of Pompeiano's group at Pisa—lead to the suggestion that sleep is not a quantitative continuum of decreasing and again increasing vigilance but that it consists of two different states of behavioural and neural organisation: the sleep with REM (REM sleep) and the sleep without REM (NREM sleep). The alteration between REM sleep and NREM

sleep occurs in all mammalian species from man to the opossum

In sleeping human infants Zipferling (150) observed as early as 1913 repeatedly occurring periods of REM with irregular respiration and body moult but the significance of this observation was not understood until Klement & Engelman (77) and Aserinsky & Klement (6) reported the finding of an alternating sleep pattern also in the very young age group. Since then meticulous behavioural observations (10 148 149) and comprehensive polygraphic investigations (25 34 35 37 38 39 40 53 91 102 103 104 108 109 110 115 116 132) have been carried out on sleeping premature and full term newborns. Fewer reports have been published about the sleep pattern of infants and older children (47 79 128 141)

### NREM SLEEP

Synonyms for NREM sleep are regular sleep (148) quiet sleep (31 101) ruhiger Schlaf (66 119) sommed calme (91) state 1 (114). In the neurophysiological literature one finds the terms synchronized sleep (68) high voltage slow wave sleep (26) telecephalic sleep (67). Both deep sleep and light sleep have been used for the description of this sleep state and it is obvious that these terms must be discarded in order to avoid misunderstandings.

During NREM sleep the infant is lying quiet except for irregularly occurring sudden generalized movements called startles. Startles are not identical with the Moro response; they occur without external stimulation and they do not consist of a regular pattern of muscular activity (114). After the newborn period startles become rare. Tonic activity can be recorded from the submental muscles; this phenomenon does not always occur especially in very young infants (41). Both respiratory rate and heart rate are slow and regular (116); the systolic blood pressure is low and stable (55). There are no movements of the eye balls after the newborn period; slow regular rolling movements

may occur at the onset of the first sleep cycle (27).

The EEG of NREM sleep undergoes marked developmental changes. Until 44–46 weeks conceptional age the dominating pattern is the so called "trace alternant" (131) consisting of longer periods with low voltage activity interrupted by periods of irregular high delta and theta waves 1–4 sec long. The flat periods which may show even a zero line EEG before 32 weeks gestational age increase in amplitude with increasing age. The "bursts" of the trace alternant are mixed with low amplitude beta activity at 32 weeks gestational age with sharp waves at 36 weeks and are made up of bilaterally synchronous delta and theta waves at term. During the second month after term the trace alternant is replaced by a sequence of EEG patterns corresponding to the NREM stages of adults classified by Dement & Kleitman (28): stage 1 any pattern between wakefulness and the appearance of 12–14 cps spindles; stage 2 low voltage EEG with spindles and occasional slow waves; stage 3 intermediate stage with high amplitude slow activity and spindles; stage 4 high amplitude delta and sub delta waves without spindles. Changes in the relative amount of NREM stages during the first years of life are mainly due to a change in the amount of spindles. This peculiar EEG pattern appears during the second month of life; spindles are longest and most frequent during the third and fourth month thereafter they decrease in amount and length until they are restricted to stage 2 at the beginning of a NREM epoch (83). This development results in a continuous decrease of stage 3 and a relative increase of stage 4 while there is little change in the amount of stage 2.

In animals (7 52 81) human adults (57) and in human infants (58 84 117 120) proprioceptive reflexes undergo no or only little depression during NREM sleep as compared to wakefulness. This is also the case for the Moro reflex in infants. Exteroceptive reflexes and the vestibulo-ocular response (14 123) on the other hand are markedly depressed or disap-



pear during NREM sleep. Nociceptive reflexes of infants, as the abdominal skin reflex or the Babinsky type foot sole reflex remain unchanged during both sleep states as does the arousal threshold to painful electrical stimuli in adults (84-113). Cortical evoked responses to stimuli of different modalities are higher in NREM sleep than in REM sleep (1-59-97-145) but this does not necessarily prove different degrees of reactivity since the amplitude of the evoked responses is partly a function of the amplitude of the background EEG.

### REM SLEEP

Synonyms for REM sleep are irregular sleep (148) active sleep (31-101) unruhiger Schlaf (119) sommeil active (91) state 2 (114) D state (56-109) paradoxical sleep (69) desynchronized sleep (68) low amplitude high frequency sleep (26) rhombencephalic sleep (67) arched sleep (68).

The most prominent feature of REM sleep is the occurrence of slow and rapid eye movements under the closed or partially opened lids. REM are numerous in newborn infants and decrease in number during the first weeks of life. In newborns they are present in an irregularly varying amount throughout the whole REM epoch after the third or fourth month of life they occur in bursts separated by periods without ocular motility. It has been suggested that form and direction of the REM might have a relationship to dream imagery (30-126) but this hypothesis has been questioned because of statistical differences between the REM and the scanning eye movements during wakefulness (64-118). The occurrence of REM moreover in newborn animals and humans refutes at least a primary relation with the subjective experience of dreaming (95-98).

Several gross body movements usually leading to a change of position may occur during an epoch of REM sleep and myoclonic twitches and small movements of the extremities and the face can be observed. The face movements

may resemble psychologically meaningful facial expressions as for instance smiling or shuddering (65-143). No tonic activity can be recorded from the submental muscles.

Respiratory rate and heart rate are higher and more irregular than during NREM sleep and may even be higher than during quiet wakefulness (116). Systolic blood pressure is also higher and shows greater fluctuations (55). Penile erections may occur though not as frequent in infants (80) as in adults (46).

The EEG of REM sleep consists of occasional small beta activity superimposed on high amplitude delta waves in premature of 30 weeks gestational age. Both types of waves disappear gradually so that at term we find a flat EEG which appears to be made up only of fast activity though spectral analysis (116) reveals a high percentage of delta and sub delta activity. No important changes occur after the second or third month of life when the visible EEG pattern consists mainly of waves in the theta range.

Other authors investigating human adults or carrying out experimental work with cats or monkeys reported identical results in REM sleep: respiratory rate, heart rate and systolic blood pressure are higher and more irregular than during NREM sleep (3-48-75-141-147). Additional physiological data, though not obtained from studies in human infants appear to be important for the understanding of the functional organization of REM sleep. In adults Mandell *et al* (87-88) found urine volume, osmolality and 17 hydroxycorticoid output to be higher in REM sleep than in NREM sleep. The oxygen consumption rate increases (18) and the intracranial pressure rises (24). From animal studies it is known that cerebral circulation is augmented by 30-50% (73-125) and cerebral temperature elevated (74-133). Proprioceptive reflexes are diminished or absent during REM sleep because of descending inhibitory influences on the fusimotor system (51-82) and on the spinal motoneurons (50-93). The firing rate of single neurons in various regions of the brain may be as high as during

wakefulness but is far more irregular (16 17 43 44 62) so that Everts (42) suggests a disorganisation of inhibitory cortical interneurons which would involve a disorganisation of the cortical unit activity during REM sleep

There is growing evidence supporting the concept that REM sleep is not a steady state. Besides "tonic" mechanisms responsible for the desynchronization of the EEG and the inhibition of tone in the antigravity muscles there are phasic components related in time to the REM bursts. Respiratory and heart rate show an additional increase in frequency and irregularity (48 118) proprioceptive reflexes which are decreased during REM sleep show an additional phasic depression during REM bursts the myoclonic twitches coincide frequently with REM bursts and are probably the result of a phasic increase of neuronal activity in the pyramidal tract (2 49). Especially important is the finding of pre- and postsynaptic inhibition occurring in various afferent pathways (8 9 13 15 21 94) because it may be causally related to the phasically occurring irregular phenomenon.

#### THE ONTOGENETIC DEVELOPMENT OF SLEEP CYCLES

In pretable premature from 24-27 weeks of gestational age Dreyfus Brissac (36) found no signs of a waking state but only atypical sleep which could not be classified as REM or NREM. By means of EEG criteria the discrimination between the two sleep states is possible at a gestational age of about 29 weeks but the typical constellation of physiological parameters defining REM sleep appears rarely before the 34th week.

Concerning the duration of the single sleep stages in newborns the data given by different authors are not quite comparable owing to differences in the criteria applied differences in recording conditions and some degree of interindividual variability (10 38 53 91 105 108 109 127). There would be agreement however about the following general facts. During

the first week of life the full term newborn sleeps 17-20 hours a day. About 12 hours are spent in REM sleep 6-7 hours in NREM sleep and the rest of the time is distributed among the different waking activities. The duration of one sleep cycle is 45-120 min. NREM epochs of 10-20 min duration and REM epochs of 20-40 min duration alternate irregularly.

Total sleep time decreases during the following months of life. Parmelee *et al* (101) found an average sleep time of 17.8 hours during the first week of life and 14.3 hours at 35 weeks. The relative amount of NREM sleep increases (22 33 127) the periodic alterations between the two sleep states become more regular. After the second month of life sleep begins with an epoch of NREM sleep followed by the first REM epoch (frequently with very few eye movements) again followed by the NREM epoch of the second sleep cycle. The NREM epochs show a gradual "building up" beginning with Dement's stage 2 followed by stage 3 and stage 4 and end abruptly usually with a gross body movement then the REM epoch begins leading gradually to the next stage 2 ("sawtooth pattern" of sleep cycles (116)).

A very similar course of development has been found in all the mammalian species studied (12 23 90 130 138 144).

#### MECHANISMS AND FUNCTIONS OF SLEEP

The very complex neurophysiology of the sleep states has recently been reviewed in an excellent article by Jouvet (72). There the interested reader will find a detailed discussion of the present knowledge about structures and mechanisms responsible for the organisation of the two sleep states. A possible monoaminergic theory of sleep has come into discussion during the last few years (70 78 99 124 146). Serotonergic neurons of the raphe complex and catecholaminergic neurons of the pontine tegmentum appear to be of crucial relevance in the triggering of the two states of sleep. Increase in brain serotonin leads to an increase in

NREM sleep and blockage of monoaminoxidase leads to an elective suppression of REM sleep whilst the amount of REM sleep can be increased by reserpine releasing monoamines at the monoaminergic terminals. This theory may be especially pertinent for the clinician since he will encounter metabolic disturbances leading to changes in the biogenic amines more often than localized lesions of brain structures responsible for the regulation of sleep states.

Reviewing the large amount of new information on sleep one does not find any completely new aspect on the function of NREM sleep. As a state in which all metabolic requirements are minimized it appears to be related to the need of the organism for rest and restitution. The function of REM sleep on the other hand is still a matter of summing though largely hypothetical discussion.

In an evolutionary theory of dreaming Snyder (140) suggests that REM sleep may have survival advantage under natural conditions by periodically decreasing the subjects threshold of arousal during sleep. The weak point of this sentinel hypothesis seems to be that a lower arousal threshold for REM sleep is by no means an established fact. The threshold of arousal is not in the first place dependent on the sleep state but on the properties of the stimulus. Even for biologically meaningful stimuli the waking threshold may be higher in REM sleep than in NREM sleep (139) and potentially dangerous stimuli seem to produce reactions independently of state (84, 13).

From a developmental point of view—taking into account the decreasing percentage of REM sleep with increasing age—Roffwarg *et al* (127) have hypothesized that the REM mechanism serves as an endogenous source of functional excitation necessary for the maturation of higher centers especially in utero and shortly after birth when exogenous stimulation is still scarce. More specifically Berger (12) regards REM as a mechanism for the establishment of the neuromuscular pathways serving binocularly coordinated eye movements. If we accept these

concepts, we are still left with the problem of the functional significance of the other phase phenomena occurring during REM sleep and of the cyclically occurring REM epochs in mature subjects—Another hypothesis (118) regards REM and the irregular fluctuations in many other parameters as the result of random noise in the CNS caused by a lack of patterning afferent input during the synchronous occurring inhibition of sensory afferents. The decrease of REM sleep with the increase of the homeostatic capacities on the brain during ontogenesis (and the slight increase of REM sleep during old age (45, 127)) would be in accordance with the hypothesis—The most recent hypothesis (100, 142) suggests that alternating periods of ocular motility and ocular quiescence may not be specific to sleep but exist over all 24 hours of a day thus representing a general activity pattern of the brain.

Though many problems remain to be solved modern research has changed the traditional concept of sleep by two essential new aspects formulated by Jouvet (71, 72): 1) Our brain, like our kidneys and heart but unlike our muscular system does not rest during sleep. On the contrary it undergoes active reorganization rather than a real inhibition, and so sleep seems to be an active phenomenon. 2) Behavioural sleep does not proceed from a single process but is the manifestation of two different states of nervous activity.

#### APPLIED SLEEP STUDIES IN PEDIATRICS

Continuous polygraphic recordings during sleep have become a new tool for the diagnosis and investigation of a variety of diseases in infants and children. Under pathologic conditions both quantitative and qualitative changes in sleep patterns may occur revealing disturbances in the complex nervous and neurochemical feedback mechanisms responsible for the coordination and homeostasis in the sleep states.

In severely damaged newborns Monod *et al* (92) found a complete lack of cyclic organization

tion of sleep. Lesser degrees of neurological abnormality will lead to abnormally frequent and irregular changes of the sleep states (85-116). Since NREM sleep is the state with the highest degree of homeostasis it is usually the first to show alterations under pathologic conditions. Its relative amount may be decreased or the coordination of the recorded parameters may be disturbed. This has been seen in infants with milder degrees of birth trauma (121) in infants of diabetic mothers (135) and in infants of heroin addicted mothers (134). A decrease of REM sleep time has been shown by Petre-Quadens *et al* (111-112) in a somewhat inhomogeneous group of mentally retarded patients. The amount of single REM was found to be decreased in autistic children (96) whilst it was increased after acute head injury. The precise time schedule after which sleep patterns develop provides the possibility to use sleep recordings for the determination of gestational age in infants (103). In certain metabolic disorders as for instance phenylketonuria (54) sleep studies may reveal changes in the EEG at a time routine EEG recordings still appear inconspicuous. It is hoped that quantitative analysis of these changes will provide a possibility to control objectively the effect of treatment. Such studies are at present carried out in our laboratory by Schulte and co-workers. The absence or decrease of sleep spindles during NREM sleep and their increase under hormone substitution are new criteria for the diagnosis and control of hypothyroidism (106-108). New information about the pathophysiology of certain forms of epilepsy has been gained from EEG recordings during the different sleep stages (89-129-137). Of interest to the child psychiatrist is the finding of Broughton (19) that the classic sleep disorders nocturnal enuresis, somnambulism and nightmare—occur preferentially during arousal from NREM sleep and are virtually never associated with the rapid-eye movement dreaming state. A wide variety of diseases causing structural or functional disturbances of the brain is awaiting further exploration by poly-

graphic sleep studies in addition to anatomical, metabolic, biochemical and psychological investigations.

It is obvious that all stimulus-response studies in young infants—neurological investigations, evoked response studies, audiometric testing (56) or psychophysiological investigations (61)—require careful monitoring of the state of the subject.

Sleep studies will provide useful information about the functional organization of the brain during normal ontogenetic development. Vice versa, the pediatrician is in the favourable position that he can use developmental changes in the sleep pattern as a tool for studying sleep mechanisms in man.

## REFERENCES

1. Akiyama Y, Schulte F J, Schultz M A & Parmelee A H. Acoustically evoked responses in premature and full term newborn infants. *Electroencephalogr Clin Neurophysiol* 26: 371, 1969.
2. Ardman A, Beriochi G & Strata P. Pyramidal activity during sleep and wakefulness. *Arch Ital Biol* 101: 530, 1963.
3. Awerzky E & Housheer T R. Respiration associated with the REM stage of human sleep. *Fed Proc* 24: 339, 1965.
4. Awerzky E & Kleitman N. Regularly occurring periods of eye motility and concomitant phenomena during sleep. *Science* 118: 273, 1953.
5. — Two types of ocular motility occurring in sleep. *J Appl Physiol* 8: 1, 1955.
6. — A motility cycle in sleeping infants as manifested by ocular and gross bodily activity. *J Appl Physiol* 8: 11, 1955.
7. Baldesera F, Broggi G & Mancini M. Mono-synaptic and polysynaptic spinal reflexes during physiological sleep and wakefulness. *Arch Ital Biol* 104: 112, 1966.
8. — Presynaptic inhibition of trigeminal afferent fibers during the rapid eye movements of desynchronized sleep. *Experientia* 22: 754, 1966.
9. Baldesera F, Cesa-Bianchi M G & Mancini M. Phasic events indicating presynaptic inhibition of primary afferents to the spinal cord during desynchronized sleep. *J Neurophysiol* 29: 871, 1966.
10. Benktzer D J. *A neurological study of newborn infants*. Hemeoema, London, 1968.
11. Berger R J. Tonus of extrinsic laryngeal muscles during sleep and dreaming. *Science* 134: 840, 1961.
12. — Oculomotor control: a possible function of REM sleep. *Psychol Rev* 76: 144, 1969.

- 13 Berlucchi G Munson J B & Rizzolatti G Changes in click evoked responses in the auditory system and the cerebellum of free moving cats during sleep and waking *Arch Ital Biol* 105 118 1967
- 14 Bernuth H v & Prechtl H F R Vestibulo-ocular response and its state dependency in newborn infants *Neuropadiatrie* 1 11 1969
- 15 Buzzi E Changes in the orthodromic and antidromic response of optic tract during the eye movements of sleep *Physiol* 8 113 1965
- 16 — Discharge patterns of single geniculate neurons during the rapid eye movements of sleep *J Neurophysiol* 29 1087 1966
- 17 Buzzi E Pompeiano O & Somogyi I Spontaneous activity of single vestibular neurons of unrestrained cats during sleep and wakefulness *Arch Ital Biol* 102 308 1964
- 18 Brebbia R D & Altshuler K Z Oxygen consumption rate and electroencephalographic stage of sleep *Science* 150 1621 1965
- 19 Broughton R J Sleep disorders disorders of arousal? *Science* 159 1070 1968
- 20 Canestrini S *Über das Sinnesleben des Neugeborenen* Springer Berlin 1913
- 21 Curli G Dietsch Spill K & Pompeiano O Inibizione presinaptica e postsinaptica nel nucleo cuneato durante il sonno *Boll Soc Ital Biol Sper* 42 640 1966
- 22 Caviness W F *Atlas of electroencephalography in the developing monkey macaca mulatta* Addison Wesley Reading Mass 1962
- 23 Chase M H Sierman M B & Clemente C D Maturation pattern of sleep and wakefulness in the kitten *Fed Proc* 24 339 1965
- 24 Cooper R & Hulme H Intracranial pressure and related phenomena during sleep *J Neurol Neurosurg Psychiat* 29 564 1966
- 25 Delange M M Castan P Cadilhac J & Passouant P Les divers stades du sommeil chez le nouveau né et le nourisson *Rev Neurol (Paris)* 107 273 1962
- 26 Dement W The occurrence of low voltage fast EEG patterns during behavioral sleep in the cat *Electroenceph Clin Neurophysiol* 10 291 1958
- 27 — Eye movements during sleep in M B Bender (ed) *The oculomotor system* Harper & Row New York 1964
- 28 Dement W & Kleitman N Cyclic variations in EEG during sleep and their relations to eye movements body motility and dreaming *Electroenceph Clin Neurophysiol* 9 673 1957
- 29 — The relation of eye movements during sleep to dream activity An objective method for the study of dreaming *J Exp Psychol* 53 339 1955
- 30 Dement W & Wolpert E The relation of eye movements body motility and external stimuli to dream content *J Exp Psychol* 55 543 1958
- 31 Dittirichova J Nature of sleep in young infants *J Appl Physiol* 17 543 1962
- 32 — Development of sleep in infancy *J Appl Physiol* 21 1243 1966
- 33 Dittirichova J & Paul K The course of wake sleep in infants *Acta Nerv Sup* 8 202 1966
- 34 Dreyfus Brissac C *Electroencephalography in infancy in F Linowich (ed) Die physiologische Entwicklung des Kindes* Springer Berlin Göttingen und Heidelberg 1959
- 35 — The electroencephalogram of the premature infants and fullterm newborn Normal and abnormal development of waking and sleeping patterns in P Kellaway & I Petersen (eds) *Neurological and Electroencephalographic Correlative Studies in Infancy* Grune & Stratton New York 1964
- 36 — Sleep ontogenesis in early human prematurity from 24 to 27 weeks of conceptual age *Develop Psychobiol* 1 162 1968
- 37 Dreyfus Brissac C Fischgold H Samson D Saint Anne Dargatzis S Ziegler T Monod N & Blanc C Veille sommeil et reactivite sensorielle chez le premature et le nouveau né *Electroenceph Clin Neurophysiol Suppl* 6 418 1956
- 38 Dreyfus Brissac C & Monod N Sleep of premature and full term neonates—a polygraphic study *Proc Roy Soc Med* 58 6 1965
- 39 Dreyfus Brissac C Samson D Blanc C & Monod N L'electroencephalogramme de l'enfant normal de moins de 3 ans *Enfance Neurol* 7 143 1958
- 40 Dubois Daleq M Contribution a l'etude du fonctionnement du systeme nerveux du nouveau né *Acta Paediat Belg* 21 257 1967
- 41 Eliet Fleischer J & Dreyfus Brissac C Le sommeil du nouveau né et du premature II Relations entre l'electroencephalogramme et l'electromyogramme mentionnant au cours de la maturation *Biol Neonat* 10 316 1966
- 42 Evarts E V Effects of sleep and waking on activity of single units in the unrestrained cat in G W W Woltzenholze & M O'Connor (eds) *The nature of sleep* Churchill London 1961
- 43 — Activity of neurons in visual cortex of the cat during sleep with low voltage fast EEG activity *J Neurophysiol* 25 812 1962
- 44 — Temporal patterns of discharge of pyramidal tract neurons during sleep and waking in the monkey *J Neurophysiol* 27 152 1964
- 45 Feinberg I & Carlson V R Sleep variables as a function of age in man *Arch Gen Psychiat* 18 239 1968
- 46 Fisher C Gross J & Zuch J Cycle of penile erection synchronous with dreaming (REM) sleep *Arch Gen Psychiat* 12 29 1965
- 47 Gabrsek V & Scherrer J Etude électrophysiologique du sommeil de l'enfant *Rev Neuropsychiatr Infants* 14 121 1966
- 48 Gassel M M Ghelarducci B Marchisavia P L & Pompeiano O Phasic changes in blood pressure and heart rate during rapid eye move

- ment episodes of desynchronized sleep in un-restrained cats *Arch Int Biol* 102 530 1964
- 49 Cassel M M Marchisavia P L & Pompeiano O Phase change in muscular activity during desynchronized sleep in unrestrained cats *Arch Int Biol* 101 449 1964
- 50 — Tonic and phasic inhibition of spinal reflexes during deep sleep desynchronized sleep in unrestrained cats *Arch Int Biol* 102 471 1964
- 51 Cassel M M & Pompeiano O Postmotor function during sleep in unrestrained cats *Arch Int Biol* 103 347 1965
- 52 Caspato S Pompeiano O & Somogyi I Supraspinal modulation of heteronymous monosynaptic and of polysynaptic reflexes during natural sleep and wakefulness *Arch Int Biol* 102 45 1964
- 53 Golde L & van Velzer C Infant sleep rhythms *Brain* 88 1043 1965
- 54 Gross H P & Schade F J Über das veränderte Spindelaktivität im Schlaf EEG bei Kindern mit Phenyketonurie *Z Kinderheilk* 105 374 1969
- 55 Gupta J M & Scopes J Observations on blood pressure in newborn infants *Arch Dis Child* 40 637 1965
- 56 Hartmann E L *The D-episode* New Engl J Med 273 30 and 31 1965
- 57 Hodar R & Delnest V Depression of electrically induced reflexes ("R reflexes") in man during low voltage EEG sleep *Electroenceph Clin Neurophysiol* 17 617 1964
- 58 Hodar R & Orbeta I H reflexes in normal human infants: depression of these electrically induced reflexes (EIRs) in sleep *Proc Soc Biol* 110 577 1963
- 59 Hrbek A Hrbek V J & Leonard H G Sociosensory stimuli and vocal evoked responses in human infants during sleep and wakefulness *Electroenceph Clin Neurophysiol* 16 397 1969
- 60 Hutt, E J Hutt C Leonard H G Bernuth H & Mansperger W J Auditory responsiveness in the human neonate *Nature* 218 888 1968
- 61 Hutt, E J Leonard H G & Precht H F R Psychophysiological studies in newborn infants in B Saunders & L Iqvist (ed.) *Advances in child development and behavior* vol IV Academic Press New York 1969
- 62 Hutterlocher P R Evoked and spontaneous activity at early onset of spinal brain stem during natural sleep and waking *J Neurophysiol* 24 451 1961
- 63 Jacobson A Kalos A Lehmann D & Hordemaker F E Muscle tone in human subjects during sleep and dreaming *Exp Neurol* 10 418 1964
- 64 Jannet M Moiré J & Jouvet M Etude de la motricité squelettique au cours de la phase paradoxale du sommeil chez le chat *Electroenceph Clin Neurophysiol* 18 554 1965
- 65 Joppich G & Michaels R Über zerebrale Aktivitäten bei Neugeborenen und jungen Säuglingen *Dtsch Med Wochschr* 92 2295 1967
- 66 Joppich G & Schaffe F J *Neurologie des Neugeborenen* Springer Berlin Heidelberg and New York 1968
- 67 Jouvet M Telencephalic and rhombencephalic sleep in the cat in G E W Wobstenholme & M O'Connor (eds.) *The nature of sleep* Churchill London 1961
- 68 — Recherches sur les structures nerveuses et les mécanismes responsables des différents phases du sommeil physiologique *Arch Int Biol* 100 125 1962
- 69 — Paradoxical sleep—a study of its nature and mechanism in L Akers C Bally & J P Schade (eds.) *Progress in brain research* vol XVIII Elsevier Amsterdam 1965
- 70 — Somnolence et monotonie *Bull Schweiz Akad Med Wiss* 22 287 1966
- 71 — The states of sleep *Sci Amer* 216 62 1967
- 72 — Neurophysiology of the states of sleep *Physiol Rev* 47 117 1967
- 73 Kautow E Krause D & Kautow H Die Variationsform der Hirnrinde in dem Phasen desynchronisierter EEG Aktivität im natürlichen Schlaf des Katers *Arch Ges Physiol* 274 593 1962
- 74 Kawamura H Wilmoyr D I & Sawyer C H Temperature changes in the rabbit brain during paradoxical sleep *Electroenceph Clin Neurophysiol* 21 469 1966
- 75 Kohn I M & Fren E U Hemodynamic changes during sleep *J Appl Physiol* 11 867 1967
- 76 Kleitman H *Sleep and wakefulness* University of Chicago Press Chicago 1963 2nd edition
- 77 Kleitman N & Engelmann T G Sleep characteristics of infants *J Appl Physiol* 6 269 1953
- 78 Klotz W P Fulkerson A & Crozman J S The effect of parachlorophenylisotone on the sleep of the cat *Electroenceph Clin Neurophysiol* 23 481 1968
- 79 Kohler W C Loddington D & Agnew H W Sleep patterns in 2 year-old children *J Pediatr* 72 228 1968
- 80 Korner A F REM organization in neonates *Arch Ges Psychol* 19 330 1968
- 81 Kubota K Imanaga Y & Niu Y Monosynaptic reflex and natural sleep in the cat *J Neurophysiol* 28 125 1965
- 82 Kubota K & Tazaki R The foremotor activity and natural sleep in the cat *Brain Res* 3 198 1966
- 83 Leonard H G The development of sleep spindles in the EEG during the first two years of life *Neuropediatrics* 1 264 1970
- 84 Leonard H G Bernuth H v & Precht H F R Reflexes and their relationship to behavioral state in the newborn *Acta Paediatr Scand* 57 177 1968
- 85 Leonard H G & Precht H F R Polygraphische Untersuchungen bei neurologischen

- Warten Neugeborenen *Mischr Kinderheilk* 116 322 1969
- 76 Loomis A L Harvey E N & Hobart O A Cerebral states during sleep as studied by human brain potentials *J Exp Psychol* 21 127 1937
  - 87 Mandell A J & Mandell M P Biochemical aspects of rapid eye movement sleep *Amer J Psychiat* 122 391 1965
  - 88 Mandell M P Mandell A J Rubin R T Brill P Rodnick J Sheff R & Chaffey B Activation of the pituitary-adrenal axis during rapid eye movement sleep in man *Life Sci* 3 593 1966
  - 89 Martinus J Über das Verhalten epileptogener Aktivität im Schlaf beim Säugling *Mischr Kinderheilk* 116 101 1969
  - 90 Meier G W & Berger R J Development of sleep and wakefulness patterns in the infant rhesus monkey *Epilept Neurol* 12 257 1965
  - 91 Monod N & Pajot N Le sommeil du nouveau né et du prématuré I Analyse des études polygraphiques (mouvements oculaires respiration et EEG) chez le nouveau né à terme *Biol Neonat* 8 281 1965
  - 92 Monod N Elzet Fiescher J & Dreyfus Bruas C Le sommeil du nouveau né et du prématuré III Les troubles de l'organisation du sommeil chez le nouveau né pathologique Analyse des études polygraphiques *Biol Neonat* 11 216 1967
  - 93 Morrison A R & Pompeiano O An analysis of the supraspinal influences acting on motoneurons during sleep in the unrestrained cat *Arch Ital Biol* 103 497 1965
  - 94 — Central depolarization of group Ia afferent fibres during desynchronized sleep *Arch Ital Biol* 103 517 1965
  - 95 Moutet J *Les Mouvements Oculaires au cours du Sommeil Paradoxal* Thèse Imprimerie de Beaux Arts Lyon 1964
  - 96 Ornitz E M Rivilo I R Brown M B La Franchi S Parmelee T & Walter R D The EEG and rapid eye movements during REM sleep in normal and autistic children *Electroenceph Clin Neurophysiol* 26 167 1969
  - 97 Ornitz E M Rivilo I R Carr E M Panman L M & Walter R D The variability of the auditory averaged evoked response during sleep and dreaming in children and adults *Electroenceph Clin Neurophysiol* 22 514 1967
  - 98 Oswald J Slow neurophysiological swings *Ann NY Acad Sci* 138 616 1967
  - 99 Oswald J Ashcroft G W Burger R J Eccleston D Evans I I & Thacore V R Some experiments in the chemistry of normal sleep *Brit J Psychiat* 112 391 1966
  - 100 Ohmer E Hyden M P & Segelbaum R Encephalic cycles during sleep and wakefulness in humans A 24 hour pattern *Science* 164 447 1969
  - 101 Parmelee A H Sleep patterns in infants *Acta Paediat Scand* 50 160 1961
  - 102 Parmelee A H Akiyama Y Schultz M A Wenner W H Schulte F J & Stern E The electroencephalogram in active and quiet sleep in infants in P Kellaway & I Peterén (eds) *Clinical electroencephalography of children* Almqvist & Wiksell, Stockholm 1969
  - 103 Parmelee A H Schulte F J Akiyama Y Wenner W H Schultz M A & Stern E Maturation of EEG activity during sleep in premature infants *Electroenceph Clin Neurophysiol* 24 319 1968
  - 104 Parmelee A H Wenner W H Akiyama Y Schultz M A & Stern E Sleep states in premature infants *Develop Med Child Neurol* 9 70 1967
  - 105 Passouant P Cadilhac J & Delange M Le sommeil du nouveau né Considérations sur la période des mouvements oculaires *Arch Franc Pediat* 22 047 1965
  - 106 Passouant P Passouant Fontaine T & Cadilhac J L'influence de l'hyperthyroïdie sur le sommeil *Rev Neurol (Paris)* 115 333 1966
  - 107 Peiper A *Die Eigenart der kindlichen Hirnaktivität VLR Theme* Leipzig 1961 3rd edition
  - 108 Petre Quadens O Etude du sommeil chez le nouveau né normal in *Le Sommeil de l'Enfant Normal et Pathologique* Masson Paris 1963
  - 109 — On the different phases of the sleep of the newborn with special reference to the activated phase or phase D *J Neurol Sci* 3 151 1966
  - 110 — Ontogenesis of paradoxical sleep in the human newborn *J Neurol Sci* 4 153 1967
  - 111 Petre Quadens O & Jourvet M Paradoxical phase of sleep in the mentally retarded *J Neurol Sci* 3 608 1966
  - 112 Petre Quadens O Jourvet M Myte O Devos J Clara R & van Heule R Recherches sur le sommeil des oligophrènes et en particulier sur la modification du tonus musculaire *Acta Neurol Psychiat Belg* 66 575 1966
  - 113 Prano M Roridius G Rossi G F & Zamboni J Relations between threshold of arousal and electroencephalographic patterns during sleep in man *Physiol Behav* 1 55 1966
  - 114 Prechtl H F R Problems of behavioral studies in the newborn infant in C Leharman & R Hinde (eds) *Advances in the study of behavior* vol I Academic Press New York 1965
  - 115 — Polygraphic studies of the full term newborn II Computer analysis of recorded data in R Macketh & M Bax (eds) *Studies in infancy* Heinemann London 1969
  - 116 Prechtl H F R Akiyama Y Zishan P & Kerr Grant D Polygraphic studies of the full term newborn I Technical aspects and qualitative analysis in R Macketh & M Bax (eds) *Studies in infancy* Heinemann London 1968
  - 117 Prechtl H F R Kerr Grant D Lenard H G & Hrbek A The lip up reflex in the awake and sleeping newborn infant A polygraphic study *Exp Brain Res* 3 184 1967
  - 118 Prechtl H F R & Lenard H G A study of

- eye movements in sleeping newborn infants *Brain Research* 5 477 1967
- 119 — Verhaltenstypologie des Neugeborenen in F. Lüscher (ed) *Fortschritte der Psychologie* vol II Springer Berlin Heidelberg and New York 1968
  - 120 Precht H F R, Vlach V, Leonard H G & Kerr Grant D. Exteroceptive and tendon reflexes in various behavioural states of the newborn infant *Biol Neonat* 11 159 1967
  - 121 Precht H F R, Weinmann H & Akiyama Y. Organization of physiological parameters in normal and neurologically abnormal infants *Neuropadiatrie* 1 101 1969
  - 122 Proyer W. *Die Seele des Kindes*. Fernus Leipzig 1948
  - 123 Reding G R & Fernandez C. Effects of vestibular stimulation during sleep *Electroenceph Clin Neurophysiol* 24 75 1968
  - 124 Reid M, Porges C V, Stephen L M, Buxler E C & Lewis G L. The effect of reoxygenation and isoproterenol on paradoxical sleep in the monkey *Psychopharmacol* 14 12 1969
  - 125 Revnick M, Isaacs G, Everts E & Kety S. The effect of slow wave sleep and REM sleep on regional cerebral blood in cats *J Neurochem* 25 301 1968
  - 126 Roffwarg H P, Dement W C, Muzio J N & Fisher C. Dream activity: Relationship to rapid eye movements of sleep *Arch Gen Psych* 41 7 235 1962
  - 127 Roffwarg H P, Muzio J N & Dement W C. Ontogenetic development of the human sleep-dream cycle *Science* 152 604 1966
  - 128 Row J J, Agnew H W, Williams R L & Webb W W. Sleep patterns in preadolescent adults. An EEG/EOG study *Pediatrics* 47 374 1969
  - 129 Row J R, Johnson L C & Walker R. II. Spike and wave discharges during stages of sleep *Arch Neurol* 14 399 1966
  - 130 Ruckebusch J. Etude polygraphique et comportamentale de l'évolution post-natale du sommeil physiologique chez l'agneau *Arch Int Biol* 101 111 1963
  - 131 Szabo D. *EEG de prémarée jusqu'à l'âge de 5 mois et à ad à terme*. Thèse Foulon Paris 1955
  - 132 Szabo D, Delfino D, Forthomme J & Capron E. EEG of the human infant during sleep and wakefulness during the first year of life in P. Ballay & J. F. Fritschy (eds) *Neurological and electrophysiological correlates of studies on infant*. Green & Stratton, New York 1964
  - 133 Siegel T. Brain temperature of the cat during sleep *Arch Int Biol* 106 73 1968
  - 134 Schöten C. A. Alterations of the sleep cycle in heroin addicted and "abused" newborns *Neuropadiatrie* 1 89 1969
  - 135 Schöte F J, Lauer U. Part II. Nölle R. & Jürgen U. Brain and behavioural maturation in newborn infants of diabetic mothers II. Sleep cycles *Neuropadiatrie* 1 36 1969
  - 136 Schulz M A, Schulte F J, Akiyama Y & Parmelee A H. Development of electroencephalographic sleep phenomena in hypothyroid infants *Electroenceph Clin Neurophysiol* 23 351 1968
  - 137 Scollon-Lavizzani G & Hess R. Phobic stimulation during paradoxical sleep in photosensitive subjects *Neurology* 17 604 1967
  - 138 Shumay A & Harwich H F. The ontogeny of sleep in kittens and young rabbits *Electroenceph Clin Neurophysiol* 24 307 1969
  - 139 Siegel J & Langley T H. Arousal threshold in the cat as a function of sleep phase and stimulus significance *Experiments* 21 740 1965
  - 140 Snyder F. Toward an evolutionary theory of dreaming *Amer J Psychiat* 123 121 1966
  - 141 Snyder F, Hobson J A, Morrison D F & Goldfrank F. Changes in respiration, heart rate and systolic blood pressure in human sleep *J Appl Physiol* 19 417 1964
  - 142 Stern H, Parmelee A H, Akiyama Y, Schulz M A & Wenger W H. Sleep cycles characteristics in infants *Pediatrics* 43 65 1969
  - 143 Théry P C Y & Larocque J L. Phases de sommeil et sources spontanées *Acta Psychol (Amst)* 24 1 1965
  - 144 Valat J L, Jouve D & Jouve M. Evolution électroencephalographique des différents états de sommeil chez le chaton *Electroenceph Clin Neurophysiol* 17 218 1964
  - 145 Wertman E D & Kremen H. Auditory evoked responses during different stages of sleep in man *Electroenceph Clin Neurophysiol* 18 65 1965
  - 146 Wertman E D, Rapport M M, McGregor P & Jacobs J. Sleep patterns of the monkey and brain serotonin concentration. Effect of p-chlorophenylalanine *Science* 160 1361 1968
  - 147 Williams D H & Cartwright R D. Blood pressure changes during EEG monitored sleep *Arch Gen Psychiat* 20 307 1965
  - 148 Wolff I H. Observations on newborn infants *Psychosom Med* 21 110 1959
  - 149 — *The causes, controls and organization of behavior in the neonate*. Psychological Issues Vol V. Monograph 17. International Universities Press, New York 1966
  - 150 Zipperling H. Über eine besondere Form motorischer Reizzustände beim Neugeborenen (sog. Stampfen) *Z Kinderheilk* 5 31 1913

Submitted Dec 1 1969

Dept. of Paediatrics  
University of Göttingen  
Humboldtallee 38  
Göttingen  
West Germany

Key words: Infants; sleep studies



## CASE REPORT

### A CASE OF HYPERCALCEMIA AND RENAL INSUFFICIENCY IN CHILDHOOD SARCROIDOSIS?

H T LUND I TRANSBØL and I HORNUM

*From the University Clinic of Pediatrics and Medical Department A and P  
Rigshospitalet University Hospital Copenhagen Denmark.*

Sarcoidosis is an uncommon disease of childhood. Till 1956 only 113 cases had been described in the pediatric literature (6). Since then few additional cases have been published (1, 11, 13, 15). A recent review is given by Jasper & Denny (8). The disease may be encountered at any age. The youngest case reported is a 2 months old infant but 75% of the pediatric cases have occurred in the preadolescent or adolescent age groups (6, 14).

Sarcoidosis presents symptoms from several organs and tissues, the most frequent involvement being that of the lungs and the lymphatic system. The occasional occurrence of hypercalcemia is due to increased intestinal absorption of calcium which is reversed by cortisone (2, 3). Increased sensitivity to vitamin D and even to sunlight are typical features of this type of hypercalcemia (3, 16). The hypercalcemia of sarcoidosis is characterized by a rapidly progressing renal insufficiency due to formation of metastatic calcifications and by excessive hypercalcaemia. During remission of sarcoidosis, as hyperabsorption of calcium ceases, the hypercalcemia and hypercalcaemia may be maintained for months by mobilization of metastatic calcifications (7, 17).

To our knowledge only 3 cases of hypercalcemic sarcoidosis in childhood have been reported (4, 15). The following case report

describes a patient in whom symptoms of hypercalcemia and renal insufficiency were the only clinical manifestations.

## CASE REPORT

II B is a 13 year old girl whose father and two elder sibs are healthy. At the age of 18 her mother had a renal stone removed. Pregnancy, birth and postnatal development were not contributory. The patient's intake of vitamin D had always been within normal limits.

Since the age of 12 she suffered from increasing thirst, polyuria, bad appetite, fatigue and loss of weight. Five months later she was admitted to a hospital where she was found to be suffering from renal insufficiency. Urinary sediment and cultures and X ray examination of the chest and urography were normal.

On admission to the pediatric department after 1 year her general health was good. She looked pale but otherwise the physical examination was completely normal.

#### *Clinical and laboratory studies*

Examination of the blood revealed a moderate normochromic anemia and a leucocyte count of 3600/ $\mu$ l with a normal differential distribution. Thrombocyte count and clotting time were normal. SR 40 mm/hour. Serum protein 7.8 g/100 ml, gamma globulin 1.4 g/100 ml (normal range 0.6-1.10 g/100 ml), other globulins and serum albumin were normal. Rheumatoid arthritis test, antistreptolysin and antistreptolysin/hyaluronidase titers were normal. L.E. cell phenomenon was negative. Two months after BCG vaccination Mantoux I (0.1 mg tuberculin 0.1 ml) and II (0.2 mg tuberculin/0.1 ml) tests were negative. Serum bilirubin, transaminase, alkaline

PT 401 B

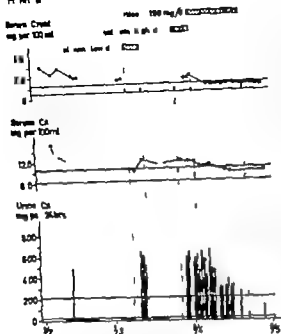


Fig. 1 Serum creatinine, serum calcium and urinary excretion of calcium during administration. Calcium low diet 100 mg calcium and 270 mg phosphorus per day. Calcium high diet 870 mg calcium and 1170 mg phosphorus per day. Cortisone 150 mg/d per 70 kg body weight (see text).

phosphatases and serum phosphorus concentrations were normal. Serum calcium values were elevated (Fig. 1).

Renal function as measured by the endogenous

clearance of creatinine and serum creatinine was decreased (Fig. 1 and Table 1). Blood pressure 120/80 mm Hg. Urinary volumes were high averaging 3000 ml/24 hour with a specific gravity below 1.010. Urinary sediment and cultures were normal. Examination for sugar and protein negative.

X-rays of the skeleton, testis, lungs, oesophagus, trachea and urinary system disclosed nothing abnormal.

Eye examination (split lamp) revealed typical band keratitis.

An open renal biopsy was performed which showed nephrocalcinosis (Fig. 2) but no glomerulomas.

Further studies of calcium and phosphorus metabolism. *Clinical course.*

On a diet containing 200 mg calcium and 270 mg phosphorus the 24-hourly urinary excretion of phosphorus was 852, 815 and 806 mg respectively. The urinary excretion of calcium was greatly increased (Fig. 1). Determinations of the phosphate excretion index (PEI) as Nordm & Frazer (12) amounted to  $+0.4$ ,  $+0.33$  (normal range  $-0.09$  to  $+0.09$ ) (Table 1). Serum calcium fractions and the tubular reabsorption of calcium ( $\text{TRCa}^{\text{m}}$ ) were measured on a standard diet as Tranbøl *et al.* (18) (Table 1). The finding of hypercalcaemia with a very low  $\text{TRCa}^{\text{m}}$  87.9% was typical of a non-parathyroid hypercalcaemia (Fig. 3).

Cortisone test as in Dent (5) by use of 100 mg of cortisone per day (150 mg per 70 kg body weight) led to normalization of serum calcium within 10 days. Also the urinary calcium excretion and serum creatinine were gradually normalized (Fig. 1 and Table 1) and urinary volumes and specific gravity became normal. Concurrently the clinical symptoms disappeared.

A paratracheal lymphnode biopsy, a bone marrow

Table 1 Serum calcium fractions, serum phosphate and the renal handling of calcium and phosphate before and during administration of cortisone

	Serum				Urine <sup>b</sup>				
	TOCa (mg/100 ml)	UFCa	Ca	P	CCr (ml/min)	Ca (mg/24 h)	P (mg/24 h)	TRCa (%)	24-h PEI
Normal range	9 10.6	6.55- 7.65	6.00- 6.60	2.5- 4.6	82 105 <sup>a</sup>	26- 224 <sup>a</sup>			-0.08- +0.09
The last two days before cortisone	12.1 12.3	9.30 9.10	8.05 7.90	3.0 3.4	34 40	589 590	840 860	87.2 88.6	+0.42 0.33
<i>Cortisone</i>									
Day 11-12	10.3	7.90	6.75	2.8	66	365	1011	95.1	+0.30
Day 13-14	10.1	7.70	7.15	2.6	71	335	992	95.7	+0.30

<sup>a</sup> TOCa = total calcium, UFCa = ultrafilterable calcium, Ca = ionized calcium.

<sup>b</sup> TRCa = the tubular reabsorption of calcium expressed as per cent of the filtered load of calcium.

<sup>c</sup> PEI = the 24-hour phosphate excretion index, CCr = the 24-hour endogenous clearance of creatinine.

<sup>d</sup> In adult subjects ( $n=83$ ).

## CASE REPORT

### A CASE OF HYPERCALCEMIA AND RENAL INSUFFICIENCY IN CHILDHOOD SARCROIDOSIS?

H T LUND I TRANSBØL and I HORNUM

*From the University Clinic of Pediatrics and Medical Department A and P  
Rigshospitalet University Hospital Copenhagen Denmark*

Sarcoidosis is an uncommon disease of childhood. Till 1956 only 113 cases had been described in the pediatric literature (6). Since then few additional cases have been published (1, 11, 13, 15). A recent review is given by Jasper & Denny (8). The disease may be encountered at any age. The youngest case reported is a 2 months old infant but 75% of the pediatric cases have occurred in the preadolescent or adolescent age groups (6, 14).

Sarcoidosis presents symptoms from several organs and tissues; the most frequent involvement being that of the lungs and the lymphatic system. The occasional occurrence of hypercalcemia is due to increased intestinal absorption of calcium which is reversed by cortisone (2, 3). Increased sensitivity to vitamin D and even to sunlight are typical features of this type of hypercalcemia (3, 16). The hypercalcemia of sarcoidosis is characterized by a rapidly progressing renal insufficiency due to formation of metastatic calcifications and by excessive hypercalcuria. During remission of sarcoidosis as hyperabsorption of calcium ceases the hypercalcemia and hypercalcuria may be maintained for months by mobilization of metastatic calcifications (7, 17).

To our knowledge only 3 cases of hypercalcemic sarcoidosis in childhood have been reported (4, 15). The following case report

describes a patient in whom symptoms of hypercalcemia and renal insufficiency were the only clinical manifestations.

## CASE REPORT

II H is a 13 year old girl whose father and two elder sibs are healthy. At the age of 18 her mother had a renal stone removed. Pregnancy, birth and postnatal development were not contributory. The patient's intake of vitamin D had always been within normal limits.

Since the age of 12 she suffered from increased thirst, polyuria, bad appetite, fatigue and loss of weight. Five months later she was admitted to a hospital where she was found to be suffering from renal insufficiency. Urinary sediment and cultures, and X ray examination of the chest and urography were normal.

On admission at the pediatric department after 1 year her general health was good. She looked pale but otherwise the physical examination was completely normal.

### *Clinical and laboratory studies*

Examination of the blood revealed a moderate normochromic anemia and a leucocyte count of 5600/ $\mu$ l with a normal differential distribution. Thrombocyte count and clotting time were normal. SR 40 mm/hour. Serum protein 7.8 g/100 ml, gamma globulin 1.4 g/100 ml (normal range = 0.62-1.10 g/100 ml), other globulins and serum albumin were normal. Rheumatoid arthritis test, antistreptolysin and antistreptohyaluronidase titers were normal. L.E. cell phenomenon was negative. Two months after BCG vaccination Mantoux I (0.02  $\mu$ g tuberculin/0.1 ml) and II (0.2  $\mu$ g tuberculin/0.1 ml) tests were negative. Serum bilirubin, transaminase, alkaline



Fig 4 Biopsy from striated muscle (femur). A multinucleated giant cell is seen surrounded by round cells (250  $\times$ )

excretion tests offer no such distinction in states of hypercalcaemia especially not when renal insufficiency supervenes (18-19).

The exclusion of hyperparathyroidism prompted the performance of additional biopsies. The changes seen in the muscle biopsy were not pathognomonic of sarcoidosis as no granulomas were found. Nevertheless the sum of pathologic findings comprising histological changes, negative Mantoux tests in spite of previous BCG vaccination, increase of the

gamma globulin fraction, elevated sedimentation rate and of course the hypercalcaemia shows a pattern suggesting sarcoidosis as the only possible diagnosis.

This case report demonstrates that hypercalcaemia should be suspected in all cases of renal insufficiency irrespective of the X-ray findings. In addition sarcoidosis should be excluded as a possible cause of unexplained hypercalcaemia even in the absence of other clinical evidence of this disease. Unnecessary



Fig. 2 Renal biopsy shows focal calcifications but no granulomatous lesions. Some of the tubules are dilated. The interstitial tissue is infiltrated with chronic inflammatory cells.

aspirate and a skin biopsy were negative. A striated muscle biopsy from the femur showed perivascular inflammatory changes and multinucleated giant cells. No granulomas were found (Fig. 4).

On treatment with prednisone 10 mg per day and a diet low in calcium and vitamin D the patient was discharged in good health for continued outpatient treatment. At control admission 2 months later she was without symptoms. Renal function, serum calcium and urinary calcium excretion were normal.

## DISCUSSION

The reported patient presents none of the classical signs or symptoms of sarcoidosis. Her complaints can all be ascribed to the hyper-

calcaemia and the accompanying renal insufficiency. Metastatic calcifications only demonstrable by slit lamp examination and renal biopsy are very frequent but not pathognomonic findings in states of non parathyroid hypercalcaemia (sarcoidosis, vitamin D intoxication, milk-alkali syndrome etc.). These calcifications are to a remarkable degree reversible. Despite hypercalcaemic symptoms for more than 1 year, correction of the hypercalcaemia is followed by a rapid normalization of renal function (7, 17). For a child the degree of hypercalcaemia is remarkable. It exceeds the levels found in a material of 57 adult cases of hyperparathyroidism (19). Also this is an important point in the differential diagnosis as in hyperparathyroidism urinary calcium excretion is generally only moderately increased or even normal in contrast to the extensive hypercalcaemia seen in many cases of hypercalcaemic sarcoidosis.

In ruling out hyperparathyroidism the measurement of the  $TRCa^*$  was valuable as was the cortisone test. The former proved to be clearly below the range of hyperparathyroidism (Fig. 3). Determination of  $TRCa$  is useful in the differential diagnosis of hypercalcaemia irrespective of the degree of renal insufficiency at least at clearances of creatinine ranging from 13 to 177 ml/min (18, 19). The phosphate

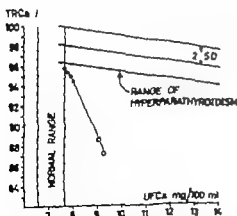


Fig. 3 The tubular reabsorption of calcium ( $TRCa$ ) related to the concentration of ultrafiltrable calcium in serum ( $UFCa$ ) in the patient before (O) and during (●) administration of cortisone. The range of hyperparathyroidism has been published previously (17).



Fig. 4 Biopsy from striated muscle (femur). A multinucleated giant cell is seen surrounded by round cells (250  $\times$ ).

excretion tests offer no such distinction in states of hypercalcemia especially not when renal insufficiency supervenes (18-19).

The exclusion of hyperparathyroidism prompted the performance of additional biopsies. The changes seen in the muscle biopsy were not pathognomonic of sarcoidosis as no granulomas were found. Nevertheless the sum of pathologic findings comprising histological changes, negative Mantoux tests in spite of previous BCG vaccination, increase of the

gamma globulin fraction, elevated sedimentation rate and of course the hypercalcemia shows a pattern suggesting sarcoidosis as the only possible diagnosis.

This case report demonstrates that hypercalcemia should be suspected in all cases of renal insufficiency irrespective of the X-ray findings. In addition sarcoidosis should be excluded as a possible cause of unexplained hypercalcemia even in the absence of other clinical evidence of this disease. Unnecessary

neck explorations have been carried out in some twenty adult patients with hypercalcemic sarcoidosis of which seven had normal X rays of the chest (20)

### SUMMARY

In a 13-year-old girl an unusual case of hypercalcemia is reported. Symptoms of hypercalcemia and renal insufficiency were the only complaints. Physical examination, and X rays were all negative. Investigation of calcium metabolism revealed hypercalcemia, excessive hypercalcaemia and discrete metastatic calcifications, discovered by slit lamp examination and renal biopsy. The tubular reabsorption of calcium (TRCa%) and the cortisone test both indicated a non-parathyroid origin of hypercalcemia. Negative Mantoux tests in spite of previous BCG vaccination, increase of the serum gamma globulin fraction, elevated sedimentation rate and a muscle biopsy showing chronic inflammatory changes and giant cells were the positive findings which rendered sarcoidosis the only probable diagnosis, although no typical granulomas were found. Finally treatment with prednisone normalized the calcium metabolic disturbances and renal function became normal.

### REFERENCES

- 1 Andersen H A. Sarcoidosis (Boeck) in a child treated with cortisone and ACTH. *Acta Paediatr Scand* 45 343 1956
- 2 Anderson J, Dent C E, Harper C & Philpot G R. Effect of cortisone on calcium metabolism in sarcoidosis with hypercalcemia. Possible actions of cortisone and vitamin D. *Lancet* II 720 1954
- 3 Bell N H, Gill J R Jr & Bartter F C. On the abnormal calcium absorption in sarcoidosis. Evidence for increased sensitivity to vitamin D. *Amer J Med* 36 500 1964
- 4 van Creveld S. Disturbances of the metabolism in Boeck's disease. *Ann Paediatr (Basel)* 137 1 1941
- 5 Dent C E. Cortisone test for hyperparathyroidism. *Lancet* I 230 1956
- 6 McGovern J E & Merritt D H. Sarcoidosis in childhood. *Advanc Paediatr* 8 97 1956
- 7 Hornum I, Transbol I & Hahnemann S. Metabolic balance and calcium 47 data in hypercalcemic sarcoidosis with and without hyperparathyroidism in J Tunaf & J Chabot (eds) *La Sarcoidose* Rapp IV conf internat Paris 1966 Masson & C edit Paris 1967 p 610
- 8 Jasper P L & Denny F W. Sarcoidosis in children. *J Pediatr* 73 499 1968
- 9 Kogut M D & Neumann L L. Renal involvement in Boeck's sarcoidosis. *Pediatrics* 28 410 1961
- 10 Knapp E L. Factors influencing the urinary excretion of calcium in normal persons. *J Clin Invest* 26 182 1947
- 11 Mandi L. Thoracic sarcoidosis in childhood. *Acta tuberc scand* 45 256 1964
- 12 Nordin B E C & Fraser R. Assessment of urinary phosphate excretion. *Lancet* I 947 1960
- 13 Scadding J G. *Sarcoidosis*. Eyre & Spottiswood London 1967
- 14 Schaud D. Die klinischen Erscheinungsformen der epithelzelliger Granulomatose im Kindesalter. *Misch Kinderheilk* 98 489 1950
- 15 Siltzbrich L E & Greenberg G M. Childhood sarcoidosis—a study of 18 patients. *New Eng J Med* 279 1239 1968
- 16 Taylor R L, Lynch H J Jr & Wysox W G Jr. Seasonal influence of sunlight on the hypercalcemia of sarcoidosis. *Amer J Med* 34 221 1963
- 17 Transbol I, Hahnemann S & Hornum I. The tubular reabsorption of calcium in the differential diagnosis between hypercalcemic sarcoidosis and primary hyperparathyroidism in J Tunaf & J Chabot (eds) *La Sarcoidose* Rapp IV conf internat Paris 1966 Masson & C edit Paris 1967 pp 616 and 645
- 18 — The tubular reabsorption of calcium in primary hyperparathyroidism and in nonparathyroid hypercalcemia. *Acta Med Scand* 184 31 1968
- 19 Transbol I, Hornum I, Hahnemann S, Hasner E, Ohlenachlger H, Diemer H & Lockwood K. The tubular reabsorption of calcium in the differential diagnosis of hypercalcemia. Further experience. *Acta Med Scand*. To be published
- 20 Winnacker J L, Becker A L & Katz S. Endocrine aspects of sarcoidosis. *New Eng J Med* 278 427 1968

Submitted Jan 1 1970

Accepted March 23 1970

(H T L)  
Falkoner Alle 36  
Copenhagen P  
Denmark

Key words: Sarcoidosis in childhood hypercalcemia

## CASE REPORT

### CONGENITAL ATRIAL FLUTTER

#### *Report of a Case*

I ANTTONAINEN and S. SIMILA

*From the Departments of Pediatrics University of Oulu, Oulu, Finland*

The term congenital atrial flutter has been used to indicate that the flutter is believed to have been present before birth and has been documented in the immediate neonatal period (4). In the literature up to 1963 Caddell & Whittemore (4) had found 10 cases which met the criteria for congenital atrial flutter. We have found in the literature another 6 case reports meeting these criteria (3-5, 7, 8).

This report of a case illustrates the clinical significance, etiological problem and therapeutic implications of this arrhythmia.

#### CASE REPORT

The patient was the first child of a healthy mother, member of the hospital staff. The mother had had measles and parotitis in her childhood. During the pregnancy she had had phlebitis in the leg. In the 4th to 28th weeks of pregnancy she had taken care of an aged patient with manifest parotitis. In the 34th week of gestation the foetal heart sounds were found to be rapid up to 200 per minute. Owing to continuing foetal tachycardia the labour was induced 4 days after the calculated term. Foetal tachycardia continued through the delivery at the rate of 130 to 100 per minute.

A girl was born on Nov. 11, 1967, in good condition with a birth weight of 3 600 g and an Apgar score of 9. In the local maternity hospital no special attention was subsequently devoted to the child. She was discharged at the age of 9 days in good condition.

For the first month the girl was well. Her gain

in weight during this time was 450 g. In the 5th week she became tired, fed poorly, coughed and vomited and was admitted to the Department of Pediatrics, University of Oulu. The girl's general condition had deteriorated. Cyanosis was noted around the mouth and her respiration was slightly laboured. The pulse rate was rapid, 160 per minute and irregular. A second-degree systolic murmur was heard on auscultation of the heart. Radiography revealed an enlarged heart, volume 410 ml/m. The electrocardiogram (Fig. 1A) showed atrial flutter; the atrial rate was 420 and ventricular rate 136 per minute. Deslanoside 0.02 mg/kg was given intramuscularly over a 24-hour period followed by a daily maintenance dose of 0.01 mg/kg for 5 days and continued with oral digoxin 0.02 mg/kg. The girl lost 300 g of weight in a fortnight but recovered.

She continued to be in good condition until at the age of 4 months she became restless and began to vomit after which she was readmitted. Her general condition had slightly deteriorated. No oedema was present. The heart rate was somewhat irregular, 160 per minute. A fourth degree systolic murmur was heard from the heart with pectus maximum in the left fourth intercostal space. The liver was palpable 3 cm below the costal margin. Radiography revealed an enlarged heart of 650 ml/m; the right atrium and right ventricle were dilated. The pulmonary vascular markings were normal. The electrocardiogram showed an atrial flutter; the atrial rate was 360 and ventricular rate 160 per minute. The treatment was increased with 0.5 mg/kg of furosemide. The child's condition improved but tachycardia continued.

The child was discharged but had to be readmitted at the age of 4 months for difficult respiration and oedema. Oedema was pronounced in the legs; the liver was palpable 6 cm below the costal margin.



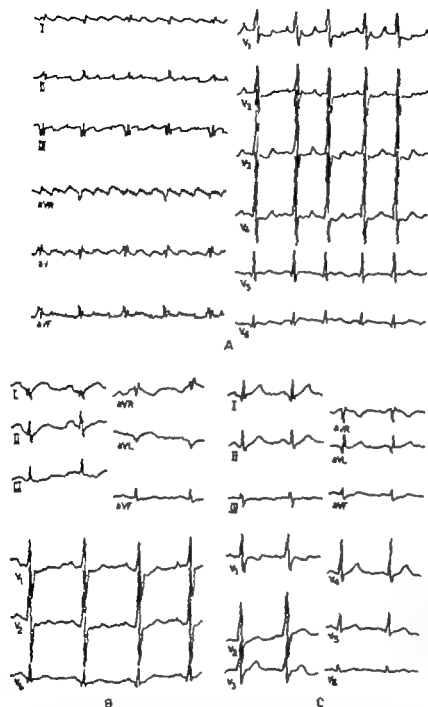


Fig 1 (A) Electrocardiogram at 5 weeks showing flutter with atrial rate of 470 per minute and ventricular rate of 165 per minute. (B) Electrocardiogram at 5 months showing a sinus rhythm. (C) Electrocardiogram at 11 months showing a sinus rhythm.

and a fifth degree systolic murmur was heard from the heart. Radiography showed a further enlargement of the heart and a slightly increased pulmonary vascularity. The ECG still revealed an atrial flutter with an atrial rate of 450 and ventricular rate of 180 per minute. In addition to digoxin (dose 0.02 mg/kg) the girl was given furosemide (dose 7 mg/kg) for 4 days. She lost 400 g of weight in 8 days and seemed better. Treatment was continued at home with digoxin (dose 0.02 mg/kg) and furosemide (dose 2.5 mg/kg).

Subsequently the girl has been well. Examination at the age of 5½ months showed no abnormal features. Her general condition was good, pulse rate 106 per minute and completely regular. A fourth

degree systolic murmur was heard from the heart. The liver was palpable 1 cm below the right margin. The ECG (Fig 1B) revealed a sinus rhythm with a rate of 110 per minute. P<sub>1</sub> was 0.35 mV, duration 0.40 sec. The dosage of furosemide and digoxin was now gradually reduced until at the age of 6 months they were discontinued. The girl was completely symptom free in good condition without any murmurs on auscultation of the heart. Radiography still showed a large heart volume, 100 ml/m. The ECG (Fig 1C) showed a sinus rhythm with a rate of 115 per minute. The P wave in II lead was 0.25 mV, duration 0.45 sec.

At the age of 13 months the girl was still symptom free. The finding on auscultation was ph

logical and the ECG still showed the sinus rhythm. Radiography revealed that the heart had diminished and its volume was 430 ml/m.

The viral isolation from throat and nasal swab specimens at the age of 5 weeks was negative. The virus antibody determinations at the age of 6 and 8 weeks showed no significant complement fixation (CF) antibody titres against cytomegalovirus, adenovirus A and B, parainfluenza 1 and 2 or respiratory syncytial viruses. The CF antibody titres against the parotitis virus at the age of 8 weeks was 1:8. The skin test with mumps antigen at the age of 6 weeks was negative. Serum immunoglobulins at the age of 2 months gave the following results: IgG 400, IgA 88 and IgM 100 mg/100 ml. Of these the IgA was high and IgM was slightly above the normal values for Finnish children at this age (4).

The virus antibody determinations carried out on the mother 6 weeks post partum showed a cytomegalovirus and parotitis antibody titre of 1:64 and as well as parainfluenza 2 titre of 1:32. The influenza A and B, parainfluenza 1 and 2, rubella, coxsackie A and B and measles titres were normal. Six weeks later the cytomegalovirus antibody titre was 1:32 and parotitis antibody titre 1:128.

## DISCUSSION

The present case meets the criteria for atrial flutter. Foetal tachycardia was noticed 4 weeks antenatally and atrial flutter was recorded electrocardiographically at the age of 5 weeks.

The etiology of congenital atrial flutter is in most cases unknown (3, 4). It may be associated with a congenital heart defect (4) or a sub-epicardial fibroelastosis (9).

The contact with parotitis during pregnancy recorded by McDonald in his 2 cases of congenital atrial flutter is very interesting (8). In our case too the mother had been in contact with parotitis in the 24th to 28th week of gestation. The high maternal CF antibody titre of mumps suggest a history of recent parotitis. This titre aroused suspicion although it was not definitely elevated.

Parotitis is apparently one of the etiological causes of fibroelastosis and in these patients a skin test with mumps antigen is often positive (12). A skin test in our case at the age of 6 weeks was negative. The only slightly increased IgM quantity (6) at the age of 10 weeks contradicts the assumption of intra-

uterine infection (1). On the basis of these findings no etiological causal connection can be critically demonstrated between the maternal parotitis and congenital atrial flutter although in our case it was highly possible. A detailed record of the history of pregnancy as regards parotitis is necessary for all patients with congenital atrial flutter in order to throw light on the etiology of this condition.

Digitalis is the drug of choice for the treatment of congenital atrial flutter. The sinus rhythm returns in 75% of the cases after 1 day to 4 months of treatment (5), most frequently within the first week (2, 3, 8, 10, 11). Cardioversion to normal sinus rhythm with D.C. fibrillation was performed in a case reported by Hassendruck *et al.* (5) without significant side effects. The method might be useful if sinus rhythm is not achieved within a week of digitalis therapy.

## SUMMARY

A case of atrial flutter which was most likely congenital is described. During pregnancy the mother had been in contact with mumps. A normal IgM quantity and negative skin test with a mumps antigen were however against the assumption of intrauterine infection and fibroelastosis. The part possibly played by parotitis is discussed. A normal sinus rhythm was achieved by therapy with digitalis and furosemide at the age of 5 months. The child has at the age of 5½ months no signs of a congenital heart defect.

## ACKNOWLEDGEMENT

We thank Doc. MUDr. Hana Padovcova CSc. for the clinical examination of this patient at the age of 13 months.

## REFERENCES

1. Allford C. A., Schaefer J., Blankenship W. J., Stramford J. V. & Cassidy G. Correlative immunologic, microbiologic and clinical approach to diagnosis of acute and chronic infections in newborn infants. *New Eng J Med* 227: 437 (1967).

- 2 Anderson R C & Adams F H Congenital paroxysmal tachycardia *J Pediatr* 43 668 1953
- 3 Blumenthal H Jacobs J C Steer C M & Williamson S W Congenital atrial flutter: report of a case documented by intra uterine electrocardiogram *Pediatrics* 41 659 1968
- 4 Caddell J L & Whittemore R Pulmonary atresia with dilated right ventricle. A case with congenital atrial flutter *Amer J Cardiol* 12 254 1963
- 5 Huxenruck A Chojnacki B & Barker H J Cardioversion of auricular flutter in a newborn infant *Amer J Cardiol* 15 726 1965
- 6 Immonen P Levels of the serum immunoglobulins gamma A gamma G and gamma M in the malabsorption syndrome in children *Ann Paediat Fenn* 13 115 1967
- 7 Landtman B & Hassula E Auricular flutter in infancy *Acta Paediat Scand* 44 272 1955
- 8 McDonagh B J Congenital atrial flutter *Arch Dis Child* 43 731 1968
- 9 Siderides L E Antonius N A & Richman A Unusual auricular flutter in newborn infant *J Pediatr* 51 435 1957
- 10 Stadler H H Prenatal auricular flutter *J Pediatr* 33 624 1948
- 11 Tarnower H & Laitin B Tachycardia in the newborn *New York J Med* 42 805 1942
- 12 Vosburgh J H Diehl A M Liu C Lauer R M & Fabry A Relationship of mumps to endocardial fibroelastosis complement fixation hemagglutination inhibition and intradermal skin tests for mumps in children with and without endocardial fibroelastosis *Amer J Dis Child* 109 69 1965

Submitted March 2 1970

Accepted March 18 1970

(I A) Department of Pediatrics  
University of Oulu  
Oulu  
Finland

**Key words** Auricular flutter congenital heart disease parotitis

## CASE REPORT

### MYELOPROLIFERATIVE DISEASE IN A CHILD WITH MONOSOMIA OF A C GROUP CHROMOSOME

J POLAK and J ZIZKA

*From the Department of Paediatrics Faculty of Medicine Hradec Králové  
Czechoslovakia*

An infant was admitted to this hospital with a leukaemoid reaction of an obscure cause in April 1963. It was followed up and several alterations were observed during nearly 4 years of duration of the disease. We believe that the course of the disease and some findings are interesting and therefore we are presenting this case.

## CASE REPORT

L. H. a girl was born on December 21 1967 to healthy parents who later gave birth to a normal male child. A disease resembling the reported one had not been observed in the family. The patient was admitted for the first time at the age of 4 months (on April 16 1963) because of bronchopneumonia. Physical examination revealed a 5 cm enlargement of the spleen and the liver 3 cm below the costal margin. The lymph nodes were not enlarged. The blood count showed a leukaemoid reaction. See Table 1. Bone marrow examination showed a granulocytic hyperplasia without other pathological findings. The child was treated with antibiotics and prednisone. The pneumonia cleared and the leucocyte count decreased to 19 500/mm<sup>3</sup> within 3 weeks. The number of nucleated red cells and immature granulocytes in the peripheral blood decreased and the hepatosplenomegaly diminished somewhat. The child was discharged in good condition after 4 weeks. Other findings during the first hospitalization were as follows: Bleeding time: coagulation time: total serum protein: paper protein electrophoresis: urinalyses: serum alkaline phosphatase: serum glutamic oxalacetate and glutamate pyruvate transaminases: serum lactic acid dehydrogenase: were within normal limits. The Paul-Bunnell reaction was negative. There were no radiological changes in the bones.

After discharge the child was followed as an out-patient. During the remaining months of the year 1963 the hepatosplenomegaly diminished slowly and the leucocyte count decreased. The good general condition of the child was not influenced by the blood disorder the way of life was not restrained and no drugs were given to treat blood diseases up to the age of 4 years. The growth the motor and mental development were fully normal till that age. Nevertheless the child was admitted to hospital three times in the years 1964 and 1965. At the age of 18 months (June 1964) a palsy of the facial nerve occurred which disappeared completely without subsequent sequelae. The cytogenetic examination of the cultured bone marrow was carried out during hospitalization in December 1964 at the age of 2 years. The findings are given separately. At that age the spleen and the liver were palpable at the costal margin the white blood count being 5 400/mm<sup>3</sup>.

In February 1965 new alterations of some mononuclear cells were noted. These cells resembled atypical reticulum cells in leukaemic reticulosis. They were observed later too but not constantly and in general their number decreased. At the age of 2 1/2 years (July 1965) the girl was admitted with acute diarrhoea vomiting acetonaemia and haematuria. She was treated with antibiotics and parenteral infusions of 5% glucose and salt solutions. Haematuria ceased and never returned and intravenous pyelography was within normal limits. The white blood count was 8 000/mm<sup>3</sup>. The temperature sedimentation rate of erythrocytes serum glutamic oxalacetate and glutamate pyruvate transaminases were normal. Neither the liver nor the spleen were palpable. The clinical remission was then complete.

In July 1966 (3 1/2 years) the myelocytes and the normoblasts in peripheral blood were again noted but neither the spleen nor the liver was palpable. The general condition of the patient was still good. In November a rash appeared consisting of small reddish itchy pimples on the skin of the trunk.

Table 1 Peripheral blood counts

Date	Years	Months	Age	Differential count															
				Erythrocytes (millions)	Retikuloeytes (%)	Leukoeytes <sup>a</sup>	Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes	Neutrophils band	Neutrophils segmented	Eosinophils	Basophils	Monocytes	Lymphocytes	Lymphocytes atypical	Reticulum cells	Nucleated red cells per 100 WBC
17.4.1963	0	3	3.06	2.0	74	200	—	1	3	8	10	13	2	—	6	37	16	—	5
6.5.1963	0	4	3.50	—	—	19	500	—	—	3	7	12	11	—	3	29	32	—	1
1.7.1963	0	6	3.38	2.2	24	100	—	—	2	4	8	24	10	—	—	30	22	—	2
13.11.1963	0	10	3.60	—	—	15	700	—	—	2	6	15	6	—	3	43	25	—	1
15.5.1964	1	4	3.80	—	—	6	700	—	—	3	3	28	3	—	5	38	18	—	2
15.12.1964	1	11	3.89	0.9	5	400	—	—	—	1	4	28	1	—	18	30	18	—	—
2.2.1965	2	1	3.80	—	—	7	100	—	—	1	4	14	—	—	29	37	15	—	—
10.7.1965	2	6	3.80	—	—	8	000	—	—	1	4	21	1	—	1	35	17	—	—
10.2.1966	3	1	3.50	—	—	4	700	—	—	—	4	33	2	—	4	43	13	—	—
26.7.1966	3	7	3.21	3.6	4	200	—	—	2	3	5	29	2	—	11	46	3	—	2
21.12.1966	4	0	3.24	—	—	4	800	5	—	1	4	29	3	5	3	27	20	—	8
4.1.1967	4	0	2.69	—	—	4	400	2	—	1	2	3	19	5	3	43	6	—	35
10.1.1967	4	0	2.11	4.6	12	500	1	—	—	1	7	39	1	2	6	27	16	—	109
18.1.1967	4	0	2.94	—	—	18	300	—	—	1	1	2	37	2	4	35	16	—	274
23.1.1967	4	1	2.82	2.8	2	400	—	—	—	—	5	68	—	—	1	19	7	—	74
4.2.1967	4	1	1.83	—	—	700	—	—	—	1	1	18	2	—	2	59	7	10	8

<sup>a</sup>Not corrected for nucleated red cells

and of the extremities. After treatment with salicyl ointment containing dexamethasone the rash disappeared and was not seen again. On December 21 1966 at the age of 4 years the liver and the spleen were palpable again 3 and 4 cm below the costal margin respectively. There was also noticed a mild enlargement of the cervical axillary and inguinal lymph nodes.

In the first week of January 1967 the number of erythrocytes fell to 2.5 millions. At the same time the number of normoblasts in the peripheral blood attained a higher level but there were no signs of significantly increased hemolysis. Tonsillitis with fever appeared and the general condition of the patient deteriorated. On January 10 1967 the child was admitted to hospital for the first time. The number of nucleated red cells rose strikingly in the peripheral blood to a maximum 224 per 100 WBC on January 18. Marked erythroid hyperplasia was noted in the bone marrow 88 cells being of the red series. The child was treated with antibiotics, transfusions, prednisone and merkaptopurine. The temperature returned to normal and the number of erythrocytes rose. However the improvement was only temporary. Towards the end of January the number of all the blood cells began to decrease rapidly. A bone marrow puncture performed on February 10 showed pronounced hypocellularity. The temperature rose again stomatitis and a bleeding tendency appeared. The parents of the patient refused further hospital care. The child was discharged and died on February 20 1967 at her home at the age of 4 years and 3 months. Autopsy was not allowed by the parents.

#### Chromosome analyses

Bone marrow for cytogenetic examination was sampled on two occasions on December 1<sup>st</sup> 1964 and on January 16 1967. The bone marrow cells were examined after 24–48 hours in culture without the use of phytohemagglutinin. The leukocytes of the peripheral blood were cultured three times with the addition of phytohemagglutinin. The cells from the first bone marrow specimen were characterized by monosomy for a C group chromosome. Cytogenetic studies of bone marrow cells in January 1967 revealed 45 chromosomes with one member of the C group missing. In addition a ring chromosome replaced a member of group C (Fig. 1). No numerical or morphological chromosome abnormalities were found in the peripheral blood cells (Table 1). The results of these examinations. Buccal smears were positive for sex chromatin. Analysis of the drumstick appendages of 500 segmented neutrophils revealed 15 cells with drumsticks.

#### DISCUSSION

The above described case has been exceptional in our practice owing to its picture and diagnosis of myeloproliferative disease. If we consider the myeloproliferative disease as not being a primary disease but only a reaction of the haemopoietic tissues to a primary condition we are lacking the primary diagnosis. The peripheral blood counts and the bone marrow

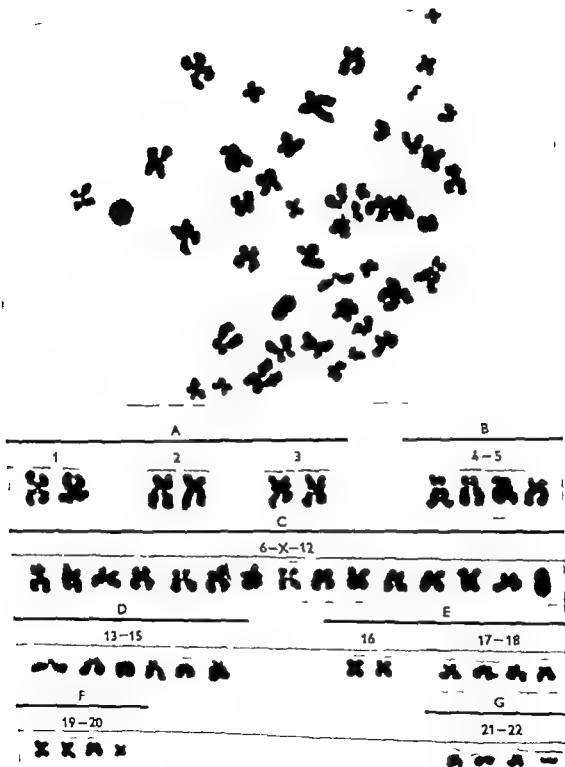


Fig. 1 Metaphase obtained from the bone marrow preparation performed on January 16, 1967 and karyotype of the same cell showing 45 chromosomes

with a ring chromosome of group C (with an arrow). One C group chromosome is missing.

Table 1 *Peripheral blood counts*

Date	Years	Months	Age	Erythrocytes (millions)	Reticulocytes (%)	Leukocytes <sup>a</sup>	Differential count													Nucleated red cells per 100 WBC	Thrombocytes	
							Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes	Neutrophils band	Neutrophils segmented	Eosinophils	Basophils	Monocytes	Lymphocytes	Lymphocytes atypical	Reticulum cells				
17.4.1963	0	3	3.06	2.0		74 700	—	—	—	1	5	8	10	15	2	—	6	37	16	—	5	1
6.5.1963	0	4	3.50			19 500	—	—	—	—	3	3	7	12	11	—	3	29	32	—	1	1
1.7.1963	0	6	3.38	2.2		24 100	—	—	—	2	4	8	8	24	10	—	3	30	22	—	2	1
13.11.1963	0	10	3.60			15 700	—	—	—	—	2	6	15	6	—	3	43	25	—	1		
15.5.1964	1	4	3.80			6 700	—	—	—	—	3	3	28	3	—	6	38	18	—	2		
15.12.1964	1	11	3.89	0.9		5 400	—	—	—	—	1	4	28	1	—	18	30	18	—	—	15	
2.2.1965	2	1	3.80			7 100	—	—	—	—	1	4	14	—	—	29	37	15	—	—		
10.7.1965	2	6	3.80			8 000	—	—	—	—	1	4	21	1	—	1	35	17	—	—		
10.2.1966	3	1	3.50			4 700	—	—	—	—	—	4	33	2	—	4	43	13	—	—		
26.7.1966	3	7	3.21	3.6		4 200	—	—	2	2	5	29	2	—	11	46	3	—	2	17		
21.12.1966	4	0	3.24			4 800	5	—	1	3	4	29	3	5	3	27	20	—	8			
4.1.1967	4	0	2.69			4 400	2	—	1	2	3	19	5	6	3	43	6	—	55			
10.1.1967	4	0	2.11	4.6		12 500	1	—	—	1	7	39	1	2	6	27	18	—	109	5		
18.1.1967	4	0	2.94			18 300	—	—	1	1	2	37	2	2	4	35	16	—	224	5		
23.1.1967	4	1	2.82	2.8		2 400	—	—	—	—	5	68	—	—	1	19	7	—	24	3		
4.2.1967	4	1	1.83			700	—	—	—	1	1	18	2	—	2	59	7	10	8	10		

<sup>a</sup> Not corrected for nucleated red cells

and of the extremities. After treatment with salicyl ointment containing dexamethasone the rash disappeared and was not seen again. On December 21 1966 at the age of 4 years the liver and the spleen were palpable again 3 and 4 cm below the costal margin respectively. There was also noticed a mild enlargement of the cervical axillary and inguinal lymph nodes.

In the first week of January 1967 the number of erythrocytes fell to 2.5 millions. At the same time the number of normoblasts in the peripheral blood attained a higher level but there were no signs of significantly increased hemolysis. Tonsillitis with fever appeared and the general condition of the patient deteriorated. On January 10 1967 the child was admitted to hospital for the last time. The number of nucleated red cells rose strikingly in the peripheral blood to a maximum 224 per 100 WBC on January 18. Marked erythroid hyperplasia was noted in the bone marrow BB cells being of the red series. The child was treated with antibiotics transfusions prednisone and merkaptopurine. The temperature returned to normal and the number of erythrocytes rose. However the improvement was only temporary. Towards the end of January the number of all the blood cells began to decrease rapidly. A bone marrow puncture performed on February 10 showed pronounced hypocellularity. The temperature rose again stomatitis and a bleeding tendency appeared. The parents of the patient refused further hospital care. The child was discharged and died on February 20 1967 at her home at the age of 4 years and 2 months. Autopsy was not allowed by the parents.

#### Chromosome analyses

Bone marrow for cytogenetic examination was sampled on two occasions on December 17 1966 and on January 16 1967. The bone marrow cells were examined after 24–48 hours in culture without the use of phytohemagglutinin. The leukocytes of the peripheral blood were cultured three times with the addition of phytohemagglutinin. The cells from the first bone marrow specimen were characterized by monosomy for a C group chromosome. Cytogenetic studies of bone marrow cells in January 1967 revealed 45 chromosomes with one member of the C group missing. In addition a ring chromosome replaced a member of group C (Fig. 1). No numerical or morphological chromosome abnormalities were found in the peripheral blood cells. Table 1 gives the results of these examinations. Baccal mean were positive for sex chromatin. Analysis of the drumstick appendages of 500 segmented neutrophils revealed 15 cells with drumsticks.

#### DISCUSSION

The above described case has been exceptional in our practice owing to its picture and diagnosis of myeloproliferative disease. If we consider the myeloproliferative disease as not being a primary disease but only a reaction of the haemopoietic tissues to a primary condition we are lacking the primary diagnosis. The peripheral blood counts and the bone marrow

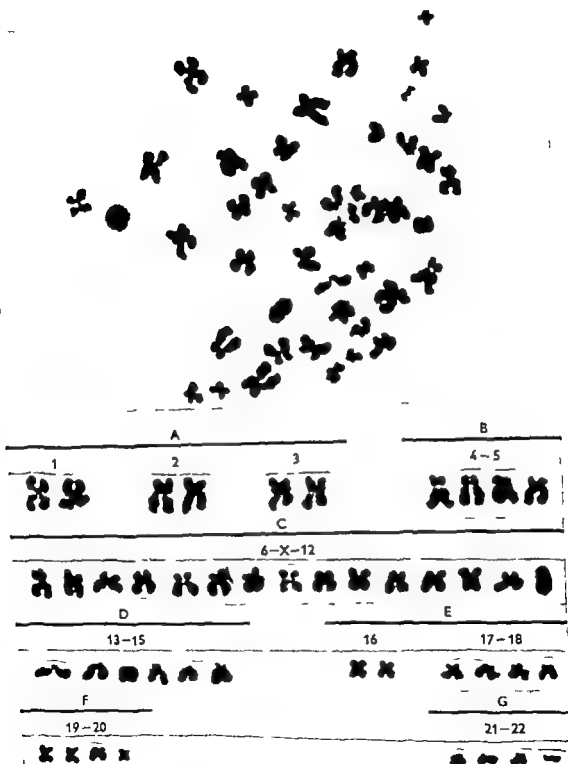


Fig 1 Metaphase obtained from the bone marrow preparation performed on January 16 1967 and karyotype of the same cell showing 45 chromosomes

with a ring chromosome of group C (with an arrow) One C group chromosome is missing



Table 2 Chromosomal findings

Date	Type of tissue	Chromosome number					Number of karyotypes				Karyotype interpretation
		41	45	46	47	P	Total	Abnormal	Normal	Total	
15.12.1964	Marrow	—	13	1	—	—	14	7	0	7	C monosomy
2.2.1965	Blood	1	3	30	—	—	34	0	3	3	Normal
8.7.1965	Blood	—	—	49	—	1	50	11	7	7	Normal
16.1.1967	Blood	—	1	9	—	—	10	0	1	1	Normal
16.1.1967	Marrow	1	23	1	—	2	27	12	0	12	C monosomy Ring chromosome of group C

findings partly the cytogenetic findings and the age exclude chronic myeloid leukaemia, chronic lymphocytic leukaemia and myeloma. However there may be a relation to acute erythraemia signs of which were observed in the preterminal phase of the disease. Polycythemia vera may also be considered as a process potentially leading to myeloproliferative disease but in the observed case neither erythrocytosis nor thrombocytosis were noted in any phase of the disease.

The relatively high number of lymphocytes and the presence of atypical lymphocytes during the leukaemoid reaction and later on proves that the pathological process has influenced not only the production of myeloid and erythroid cells but also the production of lymphocytes. The abnormalities of mononuclear cells in the relatively benign phase of the condition led us to consider the possibility of primary reticuloendotheliosis. However this was not proved by the later course of the disease.

The disease was recognized during pneumonia and it is difficult to exclude the possibility of the leukaemoid reaction being initiated by an infection. However as it occurred at the age of 4 months, it is probable that the pathological condition was inborn. Although the early course of the disease was milder and anaemia and thrombocytopenia have occurred as late as in its terminal phases, the condition has a few features in common with cases described by Randall *et al.* In their report no morphological or numerical abnormality in chromosome studies was found but the karyotype of bone

marrow cells was analyzed in one patient only.

Chromosome findings in the myeloproliferative syndrome are not uniform. There have been reported (a) numerical changes trisomy or monosomy most frequently in the C group (b) structural changes deletions or breaks of the arms of a chromosome, asymmetry between the two members of the same chromosomal pair (c) normal findings (1, 3, 4, 7, 9). The majority of chromosomal changes were found in direct bone marrow preparations. Peripheral blood cultures without the addition of phytohaemagglutinin showed also chromosomal abnormalities (1) rarely was seen normal bone marrow with pathological karyotypes in the peripheral blood (4). Speed & Lawler (8) found the same chromosomal content of the bone marrow cells on direct examination or after short term culture during the chronic phase of chronic granulocytic leukaemia.

The loss of a C group chromosome in the bone marrow cells of our patient agrees with a diagnosis of a myeloproliferative disorder. It is probable that the missing C group chromosome is an autosome; however no bone marrow cultures with <sup>3</sup>H thymidine have been done. The chromosomal findings in this patient suggest that the abnormality appears at an early stage of the disease and may persist during the course of the illness. A ring chromosome was found in the second bone marrow specimen at the stage of acute erythraemia. At that time the bone marrow was dominated by erythrocytic precursors and this finding suggests that the ring chromosome was present in

the erythrocytic precursors. Ring chromosomes has been found in acute erythroleukaemia (2).

Abnormal forms of lymphocytes were found during the disease but we have not seen any chromosomal abnormalities in the peripheral blood cultures after incubation with phytohaemagglutinin. Except for short prednisone therapy in 1964 and except for the outburst with dexamethasone in autumn 1966 the patient has received no treatment with corticoids or cytostatics up to the last chromosome analysis.

### SUMMARY

A blood disease was recognized in a 4 months old infant. It appeared first as a myeloid leukaemoid reaction with occurrence of nucleated red cells and atypical lymphocytes in peripheral blood. The disease was benign at its first stages and did not influence the normal growth and development of the patient up to the age of 4 years. Then an acute deterioration appeared with a picture of acute erythraemia and the child died with signs of pancytopenia. The cytogenetic examination showed monosomy of a C group chromosome in mitoses of bone marrow cells at the age of 2 years and in addition a ring chromosome in this group in the terminal phase.

### REFERENCES

- 1 Jackson J F & Higgins L C Group C monosomy in myelofibrosis with myeloid metaplasia. *Arch Intern Med* 119 403 1967
- 2 Krogh Jensen, M Chromosomal findings in two cases of acute erythro-leukaemia. *Acta Med Scand* 180 205 1966
- 3 Karoniglou L, A Metax W J & Dameshek W Cytogenetic studies in the chronic myeloproliferative syndrome. *Blood* 28 241 1966
- 4 Padeh, B Baum G, Schaki R & Akizis E Cytogenetic studies in proliferative disorders. *Israel J Med Sci* 1 759 1965
- 5 Randall D L, Resquist C W, Gribben J H & Robinson A Familial myeloproliferative disease. *Amer J Dis Child* 110 479 1965
- 6 Rowley J D, Blandell R J & Jacobson L O Chromosome studies in preleukemia. *Blood* 27 782 1966
- 7 Sandberg A A, Ishihara, T, Crosswhite L H & Hanschke, T S Comparison of chromosome constitution in chronic myelocytic leukemia and other myeloproliferative disorders. *Blood* 20 393 1962
- 8 Speed, D E & Lawler S H Chronic granulocytic leukaemia. The chromosomes and the disease. *Lancet* 1 403 1964
- 9 Winkelstein A, Sparkes R S & Craddock, C G Trisomy of group C in a myeloproliferative disorder. *Blood* 27 722 1966

Submitted April 18 1968

Accepted Febr 19 1970

(J P)

Dept of Pediatrics  
Faculty of Medicine  
Hradec Králové  
Czechoslovakia

**Key words:** Myeloproliferative disease chromosome anomalies

## PROCEEDINGS OF PAEDIATRIC SOCIETIES

### THE SCANDINAVIAN SOCIETY FOR PERINATAL MEDICINE

Meeting in Helsinki Jan 30 1970

Anders Rane Sumner Yaffe & Sten Orrenius (Stockholm Sweden) *Perinatal development of the hepatic microsomal drug metabolizing system*

The overall activity of the oxidative enzyme systems for drug metabolism is known to be very low in hepatic microsomes derived from fetal and newborn animals. The recent identification of the component members of the microsomal electron transport chain prompted the present study. The rate limiting factor in the oxidative drug metabolic process appears to be not the amount of cytochrome P450 (the last component of the chain) but rather the rate of the NADPH mediated reduction of this cytochrome. Measurement of these molecular components may explain why activity is low in the neonate and may also elucidate the nature of the regulator(s) of this enzyme system.

Activity of the mixed function oxidase system and its components has been measured in fetal neonatal and maternal liver and placenta both in control rabbits and following phenobarbital administration. The demethylation of aminopyrine and hydroxylation of triamcinolone and benzo(a)pyrene was minimal in fetal and neonatal liver and placenta. Activity could be induced by administration of phenobarbital to the pregnant doe. A much greater (10 fold) increase in cytochrome P450 content and activity of the NADPH mediated cytochrome reductase resulted from phenobarbital treatment. A possible explanation for these findings may be the oxygen dependence of the cytochrome system.

Pentti Jouppila & Sirkka Suono (Oulu Finland) *The influence of phenobarbitone given to toxemic and normal gravidas on the serum bilirubin concentration in newborn infants*

The decrease in the bilirubin levels caused by phenobarbitone in newborn infants is believed to be due to increased activity of glucuronyl transferase following the induction of enzymes in liver microsomes. In some studies this kind of effect of phenobarbitone has been criticized. Furthermore the bilirubin levels in babies of toxemic patients have been found to be lower than in normal cases.

In this study 30 toxemic patients received a dosage of 100 mg phenobarbitone per os daily. The patients were treated in the Department of Obstetrics and Gynecology of the University of Oulu from 5 to 20 days before delivery. Some patients received in addition, other medications, e.g. pentamyl dihydralazine, Miertal, done or sulphonamides. The control material consisted of hospitalized toxemic patients who did not receive any barbiturates. The serum bilirubin concentration of newborns was followed daily from 4 to 6 days after delivery.

The other group included 20 normal maternal care gravidas who received the same dose of 100 mg phenobarbitone per os daily from 8 to 18 days before delivery. The control material of this group comprised 30 normal gravidas who had received only iron and vitamins during the pregnancy. The bilirubin levels in the babies were followed as in the group of toxemic patients.

The toxemic and normal groups without any barbiturate therapy have been compared on the basis of daily bilirubin levels in the newborn infants in the discussion. In addition the toxemic and normal groups treated with barbiturates have been compared with the control groups.

Aino Ojala & Maila Korvanto (Oulu, Finland)  
*Oestrogen given to the mother and its effect on the bilirubin level of the newborn*

In the present study 25 mg oestradiol benzoate was given intramuscularly to 60 mothers on the day before the delivery to induce labour owing to post maturity toxemia or for some other reason. Two mothers had twin pregnancies which raised the total of the children of the series proper to 62. The bilirubin content of the blood of these babies was observed for the first 7 days of life. There was a control group of 19 mothers whose labour was induced for identical reasons but without oestrogen sensitization. This group included one mother with twin pregnancy. The total of control children was therefore 20.

In the first 3 days the bilirubin levels of the children's blood did not differ significantly but after the third day the levels were considerably higher in the children whose mothers had been sensitized with oestrogen than in the control group children. At the age of  $5\frac{1}{2}$  days for example the mean bilirubin content in the blood of the series proper was 11.82 mg/100 ml ( $SD \pm 4.49$ ) and of the control group 6.07 mg/100 ml ( $SD \pm 2.71$ ). The role of oestrogen in the conjugation of bilirubin is discussed.

clinical trials on the effect of ethanol in the treatment of threatened premature labor have been reported. In New York Fuchs used the intravenous infusions and we use the oral route of the administration of alcohol. In our treatment the loading dose consists of 60 ml of 34% alcohol followed by 30 ml dose 15 min after the initial dose which the patients are also advised to take at home on the premature beginning of regular and/or painful uterine contractions. After this loading dose the patients are treated by giving at least 3 days 30 ml of alcohol three times per day after the last signs of the threatened premature labor. The labor is significantly postponed also after premature spontaneous rupture of membranes. It seems that this treatment allows the defence mechanisms of pregnancy to recover if the failure in the basic protection is reversible.

The contraindications for the treatment are disturbances in the maternal liver function and the progression of labor over 3 cm of cervical dilatation.

Pekka H. Pikkiläinen & Niels C. R. Røhde (Helsinki, Finland) *The development of alcohol dehydrogenase and its isoenzymes in the human liver*

Ref. *Nature* 222 563 1969

Kai O. Lindros & Niels C. R. Røhde (Helsinki, Finland) *Development of some enzymes involved in gluconeogenesis in human liver*

Ref. *Annals Med Exp Fenn* 47 146 1969

Tapani Louhimen (Helsinki, Finland) *Effect of ethanol on uterine contractions*

After the observation of Fuchs & Wagner 1963 that alcohol inhibits the release of oxytocin in puerperium the results of two

S. Obenshain, K. King, P. Adam, K. Rønne, H. Teramo, N. Røhde & R. Schwartz (Cleveland, USA and Helsinki, Finland) *Human fetal in situ response to sustained maternal hyperglycemia*

Ref. *Pediatric Research* 3 380 1969

Gerhard Gennser & Erik Nilsson (Lund, Sweden) *Regulation of plasma glucose level in human midterm fetus*

Earlier investigations have demonstrated a considerably lower fetal than maternal plasma glucose level in several mammalian species. Several recent works seem to indicate a different fetomaternal relationship at midgestation in homo. This communication describes determinations of plasma glucose concentration in 30 midterm human fetuses. Successive blood samples were obtained from the umbilical vessels after clamping of the cord. The average plasma glucose concentration in one umbilical artery within 60 sec after incision of the uterus was 49 and the simultaneous level in a peripheral maternal vein was 86 mg per 100 ml. At 3 min after obstruction of the umbilical flow the fetal plasma glucose level had increased markedly the change being up to 28 mg per 100 ml. The instantaneous veno-arterial difference in the umbilical plasma glucose concentration approximated 10 mg per 100 ml at the beginning of the asphyctic period. The effect of asphyxia on the fetal glycogenolytic liver enzymes was studied. The role of the placenta and the ability of the midterm fetus to participate in the regulation of its plasma glucose concentration is discussed.

H Ekelund, Ulla Hedner & B Astedt (Malmö Sweden) *Fibrinolytic activity in the human fetus*

The material consisted of blood obtained via a catheter in the umbilical artery or by heart puncture, from 74 fetuses 10–32 cm long and extracted on legal grounds from healthy women in the 12th to 24th week of pregnancy.

*Laboratory studies.* Determinations were made of fibrinolytic activity on fibrin plates (18 fetuses), euglobulin clot lysis time (8) fibrinolytic split products in serum (49) and in serum with addition of epsilon amino caproic acid (EACA) (54), fibrinogen (17), plasminogen (63), antiplasmin (31),  $\alpha$  macroglobulin

(42), inhibitors of urokinase activation by plasminogen (19).

*Results.* The fibrinolytic activity was high in all the cases studied. The plasminogen content was about 25%  $\alpha$  macroglobulin about 40% and the fibrinogen 25–30 of adult values. Inhibitors of plasminogen activation were increased and antiplasmin was of adult level. Statistical analysis showed that only  $\alpha$  macroglobulin increased with the size of the fetus.

The results show that the fibrinolytic activity in foetal blood is considerable and that the various factors of the fibrinolytic system develop early. The factors presumably form in the foetus and are necessary for maintenance of a patent fetoplacental circulation. The findings also argue against the assumption that the hyaline membrane syndrome in prematures is due to a primary deficiency of the fibrinolytic system. Defects in the fibrinolytic system in infants with the hyaline membrane syndrome are presumably secondary to other metabolic disorders.

The investigation supports rather the clinical and experimental observations showing that the fibrinolytic activity is increased in hypoxic newborns. Part of the cause of intracranial haemorrhage in these infants might therefore be explained by hyperfibrinolysis and not by a defective coagulation system. Prophylactic treatment with EACA of the infants at risk or via treatment of the mother therefore appears to be well founded.

Bengt Bengtsson, Erik Nilsson & Gerhard Gennser (Malmö Sweden) *Sodium and potassium in human fetal and maternal erythrocytes.* Determinations were made of the sodium, potassium and water content of plasma and washed erythrocytes in blood from human midterm fetuses, newborns, their mothers and from 1-day old infants.

No significant differences in the plasma sodium concentration was found between any

no of the five groups of samples. Plasma potassium in successive samples from the umbilical artery in midterm fetuses rose with time after interference with the umbilical circulation. The difference between mid term fetal and maternal plasma potassium extrapolated back wards to normal intrauterine conditions i.e. to the moment before opening of the uterus was less than  $\pm 0.5$  mE/l.

Mean intracellular potassium concentration in the five groups of erythrocytes studied varied between 93.5 and 99.5 mE/kg. No significant difference was found between any of the five groups. Sodium concentration was higher in erythrocytes from newborn infants and 1 day-old infants than in the other three groups.

It is concluded that the fetal plasma potassium concentration at mid term closely reflects the maternal level and that red blood cells from human fetuses at mid term and at term are capable of generating ionic gradients across the cell membrane of the same magnitudes as the adult red blood cells.

*Björn Westin (Danderyd, Sverige) Human fetal response to intrauterine pure tone stimulus*

Studies of the fetal response to acoustic stimuli are of interest for several reasons and are at present concerned with the following questions: 1) At what gestational age does the first response occur? 2) Are such fetal responses a measure of the function of the cochlea and its sensory end organ? 3) How do intrauterine responses compare with postnatal auditory tests? 4) Can hearing impairment be detected already in the fetus? 5) Can acoustic stimulation of the fetus evoke a cerebral response? 6) Do fetal responses to acoustic stimuli occur in other species? 7) Are fetal responses to acoustic stimuli of adaptive value? — If so it would be interesting to test this in the human fetus.

An external bell shaped plexiglass vibrator in direct contact with the abdominal wall via a liquid interface presented during 1 sec a tone

at the ear of the fetus with a frequency of 3 000 Hz (to exclude vibration sense) and a SPL of 110 dB according to intrauterine calibrations. Fetal heart rate was recorded with phonocardiogram. The presentation of the pure tone was regularly followed by tachycardia and thereafter often by bradycardia before normalization took place (Johansson Wedenberg & Westin 1963). Significant tachycardia following sound stimulation was seen in occasional fetuses in the 22nd week (corresponds well with the development of the cochlea and the sensory end organ) in about 50% of fetuses in the 26th week and in all cases in 28–36 week from estimated conception (Westin 1968). Postnatal auditory tests were consistent with normal hearing in all cases. Auro-palpebral reflex 110 dB SPL. Awakening from sleep 70 dB SPL. 500–4 000 Hz (22 cases).

A high risk material (suspected hereditary deafness) was examined in a similar fashion. Thirty fetuses were examined. The intrauterine response was normal in all cases but one. Similar results were obtained when postnatal auditory tests were performed. The fetus that showed no intrauterine response also disclosed hearing impairment postnatally (Johansson Wedenberg & Westin 1970).

Responses to acoustic stimuli can be of adaptive value. Gottlieb (1963) has indicated that unhatched young of the wood duck respond to the quacks of the mother with increases in heart rate. Shortly after they have all hatched the mother wood duck will leave the nest and make quacking calls to the ducklings in the nest whereupon all the young jump out of the nest and into the water several feet below them. This is a behaviour that has no precedent — About 24 hours before hatching the heart beat of the ducklings begins to increase in response to the quack of the mother. The increase in heart rate becomes stronger and stronger until just prior to hatching. Apparently the ducklings become imprinted to the quack of the parent while they are still in the egg (cf Kramer 1968).

Recently Sakai, Arayama & Suzuki 1969

have recorded the cerebral evoked to acoustic stimuli from the abdominal wall at the 32nd to 38th week of pregnancy. A bone vibrator and averaging electronic computer technique was used. The typical wave form of the response consisted of four prominent deflections. These deflections correspond to the well recognized slow vertex potential to auditory stimulus in young children.

Whether the human fetal response to acoustic stimuli is of adaptive value or can be used in improving learning ability remains to be tested.

*Ritva Hilty Håns Johnell & Bo A Nilsson (Uppsala, Sverige) Oxygen, carbon dioxide tension and pH in amniotic fluid during maternal hyperoxia and hypoxia in the second trimester*

*Göran Zador (Falun, Sweden) Continuous recording of the fetal heart rate and labour activity with the cardiocograph 8020 A. Experiences of one year's use*

Since December 1, 1968, the Cardiocograph 8020 A manufactured by the Hewlett Packard Company has been used at the Department of Obstetrics and Gynecology in Falun.

During a period of 1 year from December 1, 1968, we have carried out 102 monitoring, selected from 1995 deliveries. The frequency of monitoring was consequently 5.1%. The system was completed in April, 1969, with scalp electrode for fetal ECG used after the rupture of the membranes.

The recorded cases were chosen either because of their belonging to the group high risk pregnancy—first of all overcarriage and toxemia—or because of irregularity of the fetal heart rate during delivery.

Our aim has been to judge the practical value of this Cardiocograph on the level of a Swedish Central Hospital where relatively few specialists have to face a great number of

pathological labour conditions and, therefore, all technical means are of great importance.

The results have been analysed according to the investigations of Hon Caldeyro Barcia & Hammacher and have been compared with the Apgar score of the newborns one minute after the delivery.

Among the 102 recordings, 25 pathological patterns have been found. Two cases of alien pattern have been observed. In both cases the mothers received Ketobermidon chloride (ketogin) before the monitoring was started. Eight registrations of Dips type I were obtained. Seven of the mothers were treated conservatively, but all the newborns received at least Apgar score 8, which points out that this pattern appears to be harmless. In one case with mainly Dips I but also with a few Dips II we carried out Caesarean section. An extremely short umbilical cord was found during the operation.

Dips II has been found in 15 cases, five times together with tachycardia. Only three of the newborns had an Apgar score of 8 or more. To this group belonged the only stillborn child in the whole material in spite of the fact that Caesarean section was performed. In this group, another 5 Caesarean sections and 5 vacuum extractions were made. Among the newborns we found 6 cases with Apgar score 5–7 and 5 cases with Apgar score less than 5. Three cases of placental insufficiency were verified by pathological examination.

According to our preliminary results, the method is of great value by giving us increasing possibilities to distinguish alternation of fetal heart rate patterns with good or poor prognosis for the newborn. The conventional auscultation does not allow us to make qualitative estimations of any kind.

*Antti Korvikkö (Turku, Finland) Experimental hypothermia in neonatal lambs*

Nine neonatal lambs were anaesthetized with chloralose to prevent shivering during artificial

**hypothermia** Four lambs which were given the anaesthetic for sedation only shivered when sex blood cooled. The oxygen consumption, arterial output and heart rate of the non shivering lambs decreased exponentially as the temperature decreased. Oxygen consumption of the shivering animals increased from the basal level and was maintained at a clearly higher level than in non shivering lambs during cooling. The cardiac outputs decreased to the same extent in the shivering lambs as in the non shivering lambs.

The latter finding is at variance with that obtained in experiments with adult dogs (Proc 1949) in which shivering during hypothermia caused an oxygen consumption and also an increased cardiac output. The unresponsiveness of the cardiac output of neonatal lambs to increased oxygen consumption resembles the poor response of neonatal lambs to arterial hypoxia as compared to the response of adult sheep (Korvikko 1969). The basic reason for this phenomenon remains to be determined.

**Gunnlaugur Snaedal & Gunnar Bæring (Reykjavik, Iceland) Prevention of Rh isommunization in Iceland**

When it became apparent in 1968 that isommunization to the Rhesus factor could be prevented by injection of anti D antibody preparations for prophylactic measures on a nation wide basis in Iceland were instituted.

A decision was made to treat all nonsensitized Rh negative women multiparous as well as primiparous. Furthermore it was decided to include in the programme all Rh negative women with legal and spontaneous abortions as far as they could be reached.

With a total population of 203 000 it was felt that such prophylactic measures could be accomplished with relative ease.

Approximately 700 Rh negative women are expected to deliver per year in the near future. This again means approximately 450 injections of anti D immune globulin per year to effect a good Rh prophylaxis in the country.

The bloodbank in Reykjavik in close co-operation with the University Hospital maternity clinic will be the center from which the Rh prophylaxis will be directed.

All blood typing of pregnant women in the country will be performed at the bloodbank in Reykjavik which thus gains information concerning all Rh negative pregnant women in the country at each time. Those who prove to be Rh negative will be followed with antibody screening tests in the later part of pregnancy.

When Rh negative nonsensitized women deliver Coombs test and bloodtyping for Rh are performed on placental blood and those women who can benefit from an anti D immune globulin injection will receive it.

In addition to the maternity clinics in Reykjavik where approximately 50% of all deliveries in the country occur six district hospitals strategically located in different areas of the country will be equipped to do Coombs testing and Rh typing of placental blood.

In cases of spontaneous or legal abortions bloodtyping will be encouraged so that those who prove to be Rh negative can receive anti D immune globulin.

Preparations for the above-mentioned programme were completed in November 1969 and the programme was officially launched on December 17 1969.

**Matti Kauppinen, Teina Koli & Carl Erik Raaba (Helsinki, Finland) Perinatal mortality in Helsinki**

Since vaccinations and use of antibiotics have eliminated most of the mortality during childhood the perinatal mortality and the invalidating events during this period have become the central problem in pediatrics. The present perinatal mortality in Finland is about 2.5% equally late fetal death and deaths during the first neonatal week. The mortality during the first week equals the mortality during the following 783 weeks of life which makes 15 years. During fetal development and per-



natal period induced invalidity can at present time not be determined in Finland. However, Hurlitz & Redin in Östergötland, Sweden, have shown that 1.24% of the total child population between 0-15 years are invalids. More than 3/4 of these are caused by evils during prenatal and perinatal life, that is mental retardation, cerebral palsy, epilepsy, and malformations (anatomical, chemical and immunological). 3/4 of the perinatal mortality is due to low birth weight (below 2.5 kg). Mental retardation is in 90% caused prenatally and during perinatal period (Öster). Cerebral palsy is 60 times more frequent in prematures than in babies with a birthweight over 3 kg (MacDonald) and the incidence of malformations is 10 to 20 times more frequent in prematures than in fullborn (Butler & Bonham) dependent on the birth weight.

At the prenatal clinics in Helsinki with about 7 000 to 9 000 pregnancies a year the perinatal mortality has decreased from 2.6-2.7% during the period 1955-59 to 1.8% 1960-1963, 1.6% 1964-66 and to 1.2% 1967 in the group participating in the heart volume measurements. Pregnant women are advised to rest horizontally some hours daily if the heart volume is small. This group consists of about 50 000 pregnancies. In the group in which participation in this service is refused the perinatal mortality during the period 1960-1967 has been 3.5%, the number of pregnancies in the group is about 10 000.

The decrease in the perinatal mortality depends mainly on declining number of small premature babies. This development and the distribution of the cases in different groups known as associated with prematurity and low birth weight will be discussed.

Mike Kellum, Johan Gentz & Bengt Persson (Stockholm, Sweden). *Catecholamine induced lipolysis and calorigenesis in newborn piglets*

The relationship between catecholamine induced lipolysis and calorigenesis was studied in

neonatal piglets (5 hours to 14 days old) in order to define the contribution of norepinephrine (NE) to the thermogenic response of the neonate. Unlike human infants, piglets lack brown fat and therefore allow differentiation of the roles of brown and white fat in this response.

NE infusion into piglets for 25 min ( $20 \mu\text{g}/\text{kg}/\text{min}$ ) resulted in a small (mean 5.9%), variable (from -2.2 to 15.2%) increase in oxygen consumption ( $\text{VO}_2$ ) that was not correlated with the increases in free fatty acids and glycerol nor with those of glucose or lactate. The increases of  $\text{VO}_2$  seen after similar doses of epinephrine were of similar magnitude. Administration of nicotinic acid prior to the second NE infusion resulted in a suppression of lipid mobilization but no decrease of the comparative stimulated increase in  $\text{VO}_2$ . Hyperglycemia and hyperlactacidemia persisted in an older (17 days) animal; however, inhibition of lipolysis did result in a fall in stimulated  $\text{VO}_2$ .

The results show that lipid mobilization may occur without increases of  $\text{VO}_2$  and *vice versa*. Lipolysis per se therefore does not appear to be a primary determinant of NE induced calorigenesis. It is suggested rather that utilization of FFA mobilized from white adipose tissue may be a more critical factor in determining the lipolytic related component of calorigenesis effect of NE and that this may change with postnatal age.

Johan Gentz & Bengt Persson (Stockholm, Sweden). *The importance of feeding for the rise in oxygen consumption during the first days of life in newborn pigs*

Factors influencing oxygen consumption ( $\text{VO}_2$ ) and rectal temperature ( $T_{\text{r}}$ ) were studied in piglets aged 0 to 18 days. Fed animals 0 to 24 hours old had significantly higher  $\text{VO}_2$  and  $T_{\text{r}}$  than comparable groups of unfed piglets. Fed animals 1 to 18 days old showed no significant

rise in  $\dot{V}O_2$  and  $T_{re}$  although there were significant increases in body weight and decreases in thermoneutral environmental temperature. Correlations between  $\dot{V}O_2$  and  $T_{re}$  measured in the zone of thermoneutrality were present in animals 1 to 18 days old but absent in newborn unfed piglets.

When sow's milk was given to previously unfed piglets there was a marked rise in  $\dot{V}O_2$  and  $T_{re}$  both correlated with the amount of milk given. The rise in  $\dot{V}O_2$  always preceded the increase in  $T_{re}$  indicating that  $T_{re}$  per se did not determine  $\dot{V}O_2$ .

It is concluded that during the first 24 hours of life food intake has a relatively greater effect on  $\dot{V}O_2$  and  $T_{re}$  than factors such as age, weight and moderate changes in the environmental temperature. Therefore comparisons of  $\dot{V}O_2$  may be misleading if the nutritional status of the animals vary. The present definition of thermoneutral zone in this period is also questioned.

B. Thalmé & H. Shelley (Oxford, England): *Body temperature and plasma free fatty acid in normal and anoxic newborn rabbits in the first hour after delivery*

Newborn rabbits delivered by Caesarean section were placed in a perspex chamber immersed in a thermostatically-controlled waterbath. Prewarmed AIR or N was flowing through the chamber. At regular intervals one newborn rabbit was removed from the chamber, the deep colonic temperature measured and a blood sample obtained by heart puncture.

The plasma FFA level increased in AIR at 31°C within 10 min but remained unchanged in a warmer environment. The deep colonic temperature fell below 35°C within 5–10 min of delivery. Newborn rabbits delivered into N at 31°C showed a similar rate of cooling, but no rise in plasma FFA. Glucose given intraperitoneally resulted in a normal FFA rise and injection of lactic acid gave a similar but nonsignificant lower rise. The plasma insulin levels did not differ in AIR or N and no correlation was obtained between insulin and FFA.

The postnatal rise in FFA in cool AIR and the absence of this response in N could be due to the depressant effect of anoxia on the central nervous system.

Niels Rauha

## BOOK REVIEWS

H. Olbing *Harnwegsinfektion und Harnbefund bei Kindern* Georg Thieme Verlag Stuttgart 1969 112 pp DM 34.—

The title of this book is somewhat misleading as its contents is limited to a survey of different methods of diagnosing urinary tract infections by examining urine. Bacteriological diagnostics are penetrated and the value of two screening-methods (TTC test and nitrite test) is estimated. The author discusses what there is to be gained in looking for leucocytes, erythrocytes, cylinders and proteins in urine. Like Briude *et al* (Brit Med J 1967) he is of the opinion that the number of false diagnoses can be diminished by making different methods of urine examinations complementary to each other.

The book is small covering 89 pages. Its ambitious and extensive literature list containing 631 references is perhaps the greatest merit of the book.

Lennart Richard

Werner Isler *Akute Hemiplegien und Hemisindrome im Kindesalter* Georg Thieme Verlag Stuttgart 1969 207 pp DM 69.—

This book gives a comprehensive study of the acute hemisyndromes in childhood. The author describes 114 patients, 80 with thorough case reports, the other in tabulated form. About half the cases are illustrated with excellent X-ray pictures and explaining sketches

of the lesions. Unfortunately the very detailed descriptions make the book inadequate in surveyability. Each subgroup is described but there is no survey of differential diagnosis which would have been very helpful in this difficult syndrome where often prompt diagnosis is important. It is quite clear from reading this book that much can be done by modern radiology to throw light upon the acute isolated hemiplegia of obscure origin.

Ingrid Björk

Th. Brummann & H. Frey (eds) *Epilepsie im Kindesalter* Pädiatrische Fortbildungskurse für die Praxis Vol. 26 S. Karger Basel und New York 1969 136 pp DM 33.—

This book about Epilepsy in Childhood is edited by E. Rossi, the well known pediatric professor of Bern. Many authorities are behind the ten chapters—six in German and one in French. Of great clinical value are the perspicuous papers about anti-epileptic therapy and about the side effects of anti-epileptic drugs. For the more theoretically interested there are papers dealing with genetic and pathophysiological problems of child epilepsy. The contents of the book seems to be quite up to date.

It is a handy book with a very readable collection of papers.

Jan Lagergren

## ANNOUNCEMENT

### 1ST ARGENTINE CONGRESS OF NEONATOLOGY

1st Argentine Congress of Neonatology 1st Argentine Meeting of Pediatric Nursery will be held at Mar del Plata, Argentine December 3 to 6 1970. Main subject: The high risk newborn infant: clinical and surgical aspects.

For further information write to:  
Fundación Hospitalaria  
Secretary General  
Dr. José Ricardo Pinturo  
Uruguay 937—Capital  
Buenos Aires, Argentina

# RENAL FUNCTION DURING HYDROPENIA AND WATER DIURESIS IN CHILDREN WITH RECURRENT URINARY TRACT INFECTIONS

A APERIA U BERG and O BROBERGER

From the Department of Paediatrics S: Gorans Hospital Stockholm Sweden

Recurrent urinary tract infection is the pre dominant disease of the kidney. The majority of patients with this condition will finally recover but a few will despite intensive treatment finally develop renal insufficiency. The predisposing factors for this development are still largely unknown. In order to predict the course of recurrent urinary tract infection a definition of the specific pathophysiology is urgently needed. Previous studies on this subject (16, 17, 12) are sparse and have generally been carried out in unspecified adult materials which means that the disease might have been complicated by for instance arteriosclerotic changes. Functional studies of nonobstructive recurrent urinary tract infection in childhood have so far only demonstrated that reduction of urinary concentrating capacity often occurs in acute disease (27). It has also been shown that these functional changes will rapidly be restituted following recovery of the infection (28). Those studies have however been limited to determination of the urine osmolality after 16-17 hours of food and fluid deprivation and administration of vasopressin. The present re-

port dealing with a well defined material of children with recurrent nonobstructive urinary tract infections is therefore an attempt to define further the pathophysiology of this disease. The study has primarily been aimed to evaluate glomerular filtration rate, urinary sodium excretion and concentrating and diluting capacity. For this purpose the patients were studied during the transition from hydropenia to water diuresis.

## MATERIAL

Seventeen patients aged 3-14 years were studied. None of the patients had been submitted to selective renal functional studies 1-2 years earlier. Those studies have been reported elsewhere (4). All the patients had previous histories of urinary tract infection. The diagnosis had been confirmed at least on one occasion with urine culture. Most of the patients studied had 2-3 culture confirmed infections yearly. In no case were there any clinical or bacteriological signs of infection at least 2 months prior to the study. All the patients had normal blood pressure. None of them had azotemia, acidosis, hypocalcemia or hypopotasemia. In some of the patients there were signs or symptoms of nephropathies other than urinary tract infections.

An intravenous pyelography (IVP) was carried out in all the patients within a period of at most 1 year to the reported study. A complete description of the various types of radiological changes found in this material has already been given elsewhere (4). The patients in group I had normal IVPs (both parenchyma and pelvis evaluated). The patients in group II had renal parenchymal changes defined in Table 1. The clinical histories of the patients in group I and group II did not differ significantly.

Supported by the Swedish Research Council Grant B67 19X 2049-01A.

Abbreviations: V diuresis, CFR, glomerular filtration rate, C<sub>1</sub>/C<sub>2</sub>, % of filtered sodium excreted, T<sub>1</sub>, free water reabsorption (osmolar clearance—urine flow), C<sub>2</sub>, free water clearance (urine flow—osmolar clearance).

## BOOK REVIEWS

H Olbing *Harnwegsinfektion und Harnbefund bei Kindern* Georg Thieme Verlag Stuttgart 1969 112 pp DM 34.—

The title of this book is somewhat misleading as its contents is limited to a survey of different methods of diagnosing urinary tract infections by examining urine. Bacteriological diagnostics are penetrated and the value of two screening methods (TTC test and nitrite test) is estimated. The author discusses what there is to be gained in looking for leucocytes erythrocytes cylinders and proteins in urine. Like Braude *et al* (Brit Med J 1967) he is of the opinion that the number of false diagnoses can be diminished by making different methods of urine examination complementary to each other.

The book is small covering 89 pages. Its ambitious and extensive literature list containing 631 references is perhaps the greatest merit of the book.

Lennart Righard

Werner Isler *Akute Hemiplegien und Hemisyndrome im Kindesalter* Georg Thieme Verlag Stuttgart 1969 207 pp DM 69.—

This book gives a comprehensive study of the acute hemisyndromes in childhood. The author describes 114 patients 80 with thorough case reports the other in tabulated form. About half the cases are illustrated with excellent X ray pictures and explaining sketches

of the lesions. Unfortunately the very detailed descriptions make the book inadequate in surveyability. Each subgroup is described but there is no survey of differential diagnosis which would have been very helpful in this difficult syndrome where often a prompt diagnosis is important. It is quite clear from reading this book that much can be done by modern radiology to throw light upon the acute infantile hemiplegia of obscure origin.

Ingrid Byrre

Th Baumann & R Frey (eds) *Epilepsie im Kindesalter* Pädiatrische Fortbildungskurse für die Praxis Vol 26 S Karger Basel and New York 1969 136 pp DM 33.—

This book about *Epilepsia in Childhood* is edited by E Rosu the well known pediatric professor of Bern. Many authorities are behind the ten chapters—nine in German and one in French. Of great clinical value are the perspicuous papers about anti-epileptic therapy and about the side effects of anti-epileptic drugs. For the more theoretically interested there are papers dealing with genetic and pathophysiological problems of child epilepsy. The contents of the book seems to be quite up to date.

It is a handy book with a very readable collection of papers.

Jan Lagergren

## ANNOUNCEMENT

### 1ST ARGENTINE CONGRESS OF NEONATOLOGY

1st Argentine Congress of Neonatology 1st Argentine Meeting of Pediatric Nursery will be held at Mar del Plata Argentina December 3 to 6 1970. Main subject: The high risk newborn infant clinical and surgical aspects.

For further information write to:  
Fundacion Hospitalaria  
Secretary General  
Dr Jose Ricardo Pifeyro  
Uruguay 937—Capital  
Buenos Aires Argentina

Table 2 The glomerular filtration rate and urinary sodium excretion during hydropenia in patients with normal IVPs

Patients	GFR (ml/min/ 1.73 m <sup>2</sup> b.s.)	U <sub>Na</sub> V (μEq/min/ 1.73 m <sup>2</sup> b.s.)	C <sub>Na</sub> /C <sub>24</sub> (%)
Group II			
L J	114	136	0.89
A I	110	160	1.08
A K	100	119	0.83
J H	100	173	1.20
C H	98	158	1.25
A K F	86	114	0.93
Mean	100	143	1.03
S.E.M.	4.6	9.8	0.22
Normal adults	97	140	1.01
S.E.M.	5.3	12.5	0.09
P	0.7-0.6	0.8-0.7	>0.9

significant reduction of the GFR (range 17 to 80 ml/min/1.73 m<sup>2</sup> b.s.) In Fig. 1 the relationship between the GFR and the size of the renal parenchyma has been analyzed in this group of patients. The size of the renal parenchyma has been roughly estimated by one dimensional surface calculation. In none of the patients however was there any evidence of unusual disproportion between the dimensions. The kidney size has been calculated as percentage

Table 3 The glomerular filtration rate and urinary sodium excretion during hydropenia in patients with pathological IVPs

Patients	GFR (ml/min/ 1.73 m <sup>2</sup> b.s.)	U <sub>Na</sub> V (μEq/min/ 1.73 m <sup>2</sup> b.s.)	C <sub>Na</sub> /C <sub>24</sub> (%)
Group II			
A B	10	195	1.37
C K	90	116	1.00
A K	86	139	1.29
A E	80	112	0.98
S W	77	194	1.79
J J	72	107	1.03
E M L	67	173	1.79
I A	67	414	4.51
G B	48	80	1.00
I L L	33	147	3.36
A H	37	33	3.36

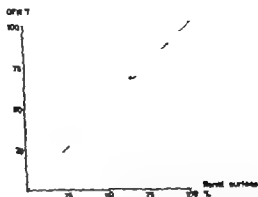


Fig. 1 The relationship between the renal surface and the GFR in all the patients with pathological IVPs. The renal surface has been calculated as percentage of the average renal surface of two normal subjects of corresponding age and sex. The GFR is also given in percentage of the normal GFR for the patients body surface. The dashed line represents the theoretical 1/1 relationship between GFR and renal surface.

of the average kidney size of two normal subjects of corresponding age and sex. The figure demonstrates a good relationship (within 15%) for 6 of the 11 patients, a complete lack of relationship in 5 of the 11 patients, one of whom having a much too high GFR, one demonstrating a 25% higher GFR than the estimated renal size and the other 3 a too low GFR. The total sodium excretion in group II was within one standard deviation of the values from the healthy volunteers in all but 1 patient (I A). The fractional sodium

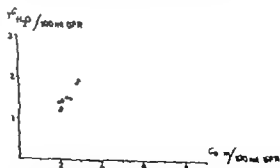


Fig. 2 The relationship between free water reabsorption ( $T^{\circ}\%$ ) and osmolar clearance ( $C_{osm}$ ) in all the patients studied. Both parameters are related to the GFR. Open circles represent the patients with normal IVPs (group I) and filled circles represent the patients with pathological IVPs (group II).

Table 1 *The radiological appearance of the kidneys in patients in group II*

Patient	Left kidney	Right kidney
C K	Moderately reduced in size Even in shape Calices normal	Moderately reduced in size Even in shape Calices normal
A K	Small in size Uneven in shape Calices deformed	Normal in size Even in shape Calices normal
G B	Moderately hypertrophied Even in shape Calices normal	Small in size Even in shape Calices deformed
J J	Moderately reduced in size Even in shape Small calcifica- tions Calices normal	Moderately reduced in size Even in shape Small calcifica- tions Calices deformed
S W	Small in size Uneven in shape Calices deformed	Normal in size Even in shape Calices normal
I L L	Small in size Even in shape Calices deformed	Normal in size Uneven in shape Calices deformed
A B	Small in size Uneven in shape Small calcifications Calices deformed	Moderately reduced in size Uneven in shape Calices normal
I A	Normal in size Uneven in shape Calices normal	Small in size Uneven in shape Calices deformed
A E	Moderately reduced in size Uneven in shape Calices deformed	Small in size Uneven in shape Calices deformed
E M L	Small in size Even in shape Calices deformed	Normal in size Even in shape Calices normal
K H	Small in size Uneven in shape Calices deformed	Small in size Uneven in shape Calices deformed

## METHODS

All the patients were studied during the transition from hydropenia to water diuresis. At the beginning of the study the patients were deprived of water for 17 hours. Following 2-3 clearance periods during hydropenia water diuresis was induced by allowing the patients to drink water in an amount corresponding to 2-2.5 of the body weight and thereafter in amounts slightly exceeding the diuresis every 30 min. When water diuresis was believed to be complete i.e. when diuresis was again constant usually within 75 to 90 min another 2-4 clearance periods were carried out. All the patients tolerated the study well and were cooperative.

Standard clearance techniques were used (24). This includes a continuous infusion of 10 mU/min (Laevasar Gesellschaft) 0.001 g/min/kg b.w. after a prime dose of 0.05 g/kg b.w. The equilibration time was 45-60 min. For urine sampling a double lumen polyethylene catheter was used enabling continuous suction. Blood samples were taken in the middle of each urine sampling period from an indwelling needle in a peripheral vein. During the hydropenic stage urine was collected in 30-45 min periods. During water diuresis the periods were of about 15 min duration.

Urine and blood samples were analyzed with regard to the concentration of inulin, sodium and osmolality. The chemical analyses of inulin were carried out according to the method of Heyrowitz (10) sodium in an Eppendorf flame photometer and osmolality cryoscopically with a Knauer microosmometer.

## RESULTS

*Glomerular filtration rate and urinary sodium excretion*

Tables 2 and 3 demonstrate the glomerular filtration rate (GFR) per 1.73 m<sup>2</sup> body surface and the urinary sodium excretion expressed in absolute values as well as in percentage of filtered load. All values are recorded during hydropenia. Table 2 contains the patients with normal IVPs (group I) and Table 3 the patients with renal parenchymal changes (group II). The patients with normal IVPs have been statistically compared to a group of healthy young volunteers. The results from the latter group have been reported in detail elsewhere (3). The patients with recent urinary tract infections but with normal IVPs did not significantly differ from the healthy volunteers with regard to the GFR, the total and the fractional sodium excretion.

The patients with pathological appearance of the IVPs (Table 3) were considered too heterogeneous with regard to the degree of renal parenchymal changes to be statistically worked up. Eight of the 11 patients demonstrate sig-

Table 2 The glomerular filtration rate and urinary sodium excretion during hydropenia in patients with normal IVPs

Patient	GFR (ml/min/ 1.73 m <sup>2</sup> b.s.)	U <sub>Na</sub> V (μEq/min/ 1.73 m <sup>2</sup> b.s.)	C <sub>Na</sub> /C <sub>Cr</sub> (%)
Group I			
L J	114	136	0.89
A J	110	160	1.08
A K	100	119	0.83
J H	100	173	1.20
C H	88	158	1.25
A E F	86	114	0.95
Mean	100	143	1.03
S.E.M.	4.6	8.8	0.22
Normal values	97	140	1.01
S.E.M.	3.3	12.5	0.09
P	0.7-0.6	0.8-0.7	>0.9

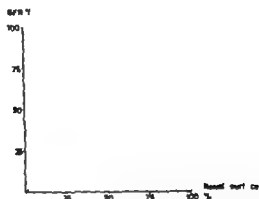


Fig. 1 The relationship between the renal surface and the GFR in all the patients with pathological IVPs. The renal surface has been calculated as percentage of the average renal surface of two normal subjects of corresponding age and sex. The GFR is also given as percentage of the normal GFR for the patient's body surface. The dashed line represents the theoretical 1/1 relationship between GFR and renal surface.

sufficient reduction of the GFR (range 17 to 80 ml/min/1.73 m<sup>2</sup> b.s.). In Fig. 1 the relationship between the GFR and the size of the renal parenchyma has been analyzed in this group of patients. The size of the renal parenchyma has been roughly estimated by one dimensional surface calculation. In none of the patients, however, was there any evidence of unusual disproportion between the dimensions. The kidney size has been calculated as percentage

of the average kidney size of two normal subjects of corresponding age and sex. The figure demonstrates a good relationship (within 15%) for 6 of the 11 patients; a complete lack of relationship in 5 of the 11 patients, one of whom having a much "too high" GFR, one demonstrating a 25% higher GFR than the estimated renal size and the other 3 a too low GFR. The total sodium excretion in group II was within one standard deviation of the values from the healthy volunteers in all but 1 patient (I A). The fractional sodium

Table 3 The glomerular filtration rate and urinary sodium excretion during hydropenia in patients with pathological IVPs

Patient	GFR (ml/min/ 1.73 m <sup>2</sup> b.s.)	U <sub>Na</sub> V (μEq/min/ 1.73 m <sup>2</sup> b.s.)	C <sub>Na</sub> /C <sub>Cr</sub> (%)
Group II			
A B	182	195	1.37
C K	90	116	1.00
A K	86	139	1.79
A E	80	112	0.98
S W	77	194	1.79
J J	77	107	1.03
E M L	67	173	1.79
I A	67	414	4.53
O B	54	80	1.00
I L L	33	147	3.36
K H	17	83	3.36

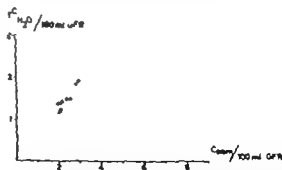


Fig. 2 The relationship between free water reabsorption ( $T^2_{H_2O}$ ) and osmolar clearance ( $C_{osm}$ ) in all the patients studied. Both parameters are related to the GFR. Open circles represent the patients with normal IVPs (group I) and filled circles represent the patients with pathological IVPs (group II).



Table 1 *The radiological appearance of the kidneys in patients in group II*

Patient	Left kidney	Right kidney
C K	Moderately reduced in size Even in shape Calices normal	Moderately reduced in size Even in shape Calices normal
A K	Small in size Uneven in shape Calices deformed	Normal in size Even in shape Calices normal
G B	Moderately hypertrophied Even in shape Calices normal	Small in size Even in shape Calices deformed
J J	Moderately reduced in size Even in shape Small calcifica- tions Calices normal	Moderately reduced in size Even in shape Small calcifica- tions Calices deformed
S W	Small in size Uneven in shape Calices deformed	Normal in size Even in shape Calices normal
I L L	Small in size Even in shape Calices deformed	Normal in size Uneven in shape Calices deformed
A B	Small in size Uneven in shape Small calcifications Calices deformed	Moderately reduced in size Uneven in shape Calices normal
I A	Normal in size Uneven in shape Calices normal	Small in size Uneven in shape Calices deformed
A E	Moderately reduced in size Uneven in shape Calices deformed	Small in size Uneven in shape Calices deformed
E M L	Small in size Even in shape Calices deformed	Normal in size Even in shape Calices normal
K H	Small in size Uneven in shape Calices deformed	Small in size Uneven in shape Calices deformed

## METHODS

All the patients were studied during the transition from hydropenia to water diuresis. At the beginning of the study the patients were deprived of water for 17 hours. Following 2-3 clearance periods during hydropenia water diuresis was induced by allowing the patients to drink water in an amount corresponding to 2-2.5 of the body weight and thereafter in amounts slightly exceeding the diuresis every 30 min. When water diuresis was believed to be complete i.e. when diuresis was again constant usually within 75 to 90 min another 2-4 clearance periods were carried out. All the patients tolerated the study well and were cooperative.

Standard clearance techniques were used (24). This includes a continuous infusion of  $10^{-4}$  insulin (Insulin Gesellschaft) 0.001 g/min/kg b.w. after a prime dose of 0.05 g/kg b.w. The equilibration time was 45-60 min. For urine sampling a double lumen polyethylene catheter was used enabling continuous suction. Blood samples were taken in the middle of each urine sampling period from an indwelling needle in a peripheral vein. During the hydropenic stage urine was collected in 30-45 min periods. During water diuresis the periods were of about 15 min duration.

Urine and blood samples were analyzed with regard to the concentration of insulin, sodium and osmolality. The chemical analyses of insulin were carried out according to the method of Heyrovsky (10) sodium in an Eppendorf flame photometer and osmolality cryoscopically with a Knauer microosmometer.

## RESULTS

*Glomerular filtration rate and urinary excretion*

Tables 2 and 3 demonstrate the glomerular filtration rate (GFR) per 1.73 m<sup>2</sup> body surface and the urinary sodium excretion expressed in absolute values as well as in centage of filtered load. All values are recorded during hydropenia. Table 2 concerns the patients with normal IVPs (group I) Table 3 the patients with renal parenchymal changes (group II). The patients with normal IVPs have been statistically compared with a group of healthy young volunteers. The results from the latter group have been reported in detail elsewhere (3). The patients with current urinary tract infections but with normal IVPs did not significantly differ from healthy volunteers with regard to the total and the fractional sodium excretion.

The patients with pathological appearance of the IVPs (Table 3) were considered heterogeneous with regard to the degree of parenchymal changes to be statistically compared.

Eight of the 11 patients demonstrate

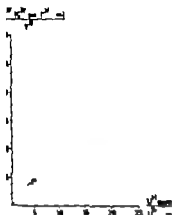


Fig 6 The relationship between expected (abscissa) and actual (ordinate) dilution of urine during the transition from hydropenia to water diuresis in the patients with pathological IVPs (group II) when differences in osmotic clearances have been corrected. Symbols see Fig 5. Those results that are beyond the normal range i.e. implying a defect in dilution have been indicated by filled circles.

had been corrected for. Since all the patients reported were studied during the transition from hydropenia to water diuresis the same analysis could be carried out with the recent results (Figs 5 and 6). It is apparent from Fig 5 that all the patients with normal IVPs are able to dilute the distal tubular fluid more during water diuresis i.e. the actual dilution of urine (ordinate) exceeds the expected dilution (abscissa) even when variance in osmolar clearance is corrected for. The results from the patients with renal parenchymal changes (group II) are however more variable (Fig 6). Five of those patients (filled circles) are apparently unable to increase their diluting capacity while the rest of the patients (open circles) respond to water diuresis in the same way as the patients with normal IVPs and the healthy adults. On the basis of those results the patients in group II have been divided into two subgroups IIa and IIb.

Changes in glomerular filtration rate and urinary sodium excretion during the transition from hydropenia to water diuresis

Table 4 demonstrates the average changes in GFR and urinary sodium excretion during the

Table 4 The mean values with one standard deviation for the glomerular filtration rate and fractional sodium excretion during hydropenia and water diuresis in the different groups. The difference of mean is also listed

	Hydropenia	Water diuresis	Mean difference
GFR (ml/min/1.73 m <sup>2</sup> b.s.)			
Group I	100 ± 11	112 ± 13	12 ± 6 $p < 0.01$
Group IIa	64 ± 28	94 ± 29	30 ± 16 $p < 0.01$
Group IIb	73 ± 26	80 ± 20	7 ± 8 $0.4 < p < 0.5$
$C_{Na}/C_{Cr}$ ( )			
Group I	1.03 ± 0.17	1.50 ± 0.33	0.48 ± 0.54 $0.05 < p < 0.1$
Group IIa	1.53 ± 0.93	2.19 ± 0.98	0.66 ± 0.69 $0.05 < p < 0.1$
Group IIb	2.47 ± 1.42	2.64 ± 1.44	0.18 ± 0.28 $0.2 < p < 0.3$

Statistically significant difference

transition from hydropenia to water diuresis in group I, IIa and IIb. The individual patterns of the GFRs are depicted in Fig 7. In the study of healthy adults the GFR was found to be slightly but significantly increased in water diuresis. The patients with normal IVPs (group I) showed the same pattern. The patients with parenchymal changes who were able to increase their diluting capacity during water diuresis i.e. belonging to group IIa also increased their GFR significantly. In fact most of these patients exhibited a steeper increase

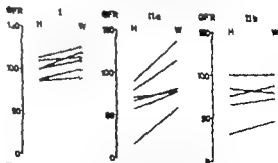


Fig 7 Changes in GFR during the transition from hydropenia to water diuresis for patients with normal IVPs (group I), patients with pathological IVPs with normal dilution (group IIa) and patients with pathological IVPs with defect dilution (group IIb).

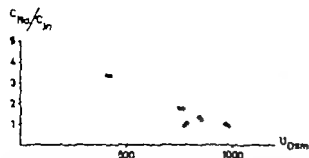


Fig 3 The relationship between fractional sodium excretion ( $C_{Na}/C_I$ ) and urine osmolality ( $U_{Osm}$ ) during maximal hydropenia. All the patients studied are included in the figure. Open circles represent the patients with normal IVPs (group I) and filled circles represent the patients with pathological IVPs (group II).

excretion was markedly increased in 3 of the patients on the upper limit in two and normal in the rest of the patients.

#### Concentrating capacity

The urinary concentrating capacity is represented both in terms of free water reabsorption and maximal urinary osmolality. In Fig 2 the free water reabsorption has been related to the osmolar clearance. All the patients (i.e. from both groups) are included in the figure. The relationship is the same as that found in healthy adults (9, 19) in all but three patients. Those 3 patients who all belong to group II have an elevation of the osmolar clearance but do not exhibit the expected increase in free

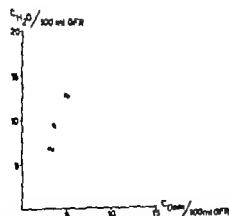


Fig 4 The relationship between free water production ( $C_{H_2O}$ ) and osmolar clearance ( $C_{Osm}$ ) during water diuresis. Both parameters are related to the GFR. All the patients studied are included in the figure. Open circles represent the patients with normal IVPs (group I) and filled circles represent the patients with pathological IVPs (group II).

water reabsorption that is found when osmolar clearance is raised with mannitol or saline diuresis (9, 19).

In order to reveal further the mechanism of reduced concentrating capacity in some of the patients the relationship between urinary osmolality and the fractional sodium excretion has been examined (Fig 3). It is quite clear that a marked increase in fractional sodium excretion is always accompanied by a marked fall in urinary osmolality.

#### Diluting capacity

Fig 4 demonstrates the diluting capacity as expressed by free water clearance in relation to osmolar clearance in all the patients studied. The values are within the range that found in healthy adults (3).

Previous studies in healthy adults have demonstrated that the fluid of the distal tubule is more diluted during water diuresis than during hydropenia (3). This finding was accomplished by comparing actual and expected dilution of urine after discrepancies in osmotic

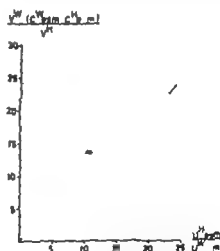


Fig 5 The relationship between expected (abscissa) and actual (ordinate) dilution of urine during the transition from hydropenia to water diuresis in the patients with normal IVPs (group I) when differences in osmotic clearances have been corrected for.  $V^H$  urine flow ml/min during water diuresis.  $V$  urine flow ml/min during hydropenia.  $U^H$  urine osmolality during water diuresis.  $U^H$  urine osmolality during hydropenia.  $C^H$  = osmolality clearance during water diuresis.  $C^H$  = osmolality clearance during hydropenia. The dashed line represents the theoretical 1/1 relationship between the expected and actual dilution of urine.

need that the diluting capacity is primarily a function of the active sodium reabsorption in the distal tubule. It has been implied by several authors that the intrarenal regulation of the blood supply is by some means linked to the sodium reabsorption at the site of the macula densa, i.e. the early distal tubule (1, 2, 7, 25, 26). Thus it has been suggested that impairment of sodium reabsorption at this site will cause a renal vasoconstriction (1, 2). It is therefore of interest that the patients that were unable further to dilute the urine i.e. increase the distal sodium reabsorption during water diuresis constitute the only group where the GFR was not significantly increased during water diuresis. Thus the results from this study are suggestive of a change in the renal vascular tone secondary to subtle changes in the tubular handling of sodium in some of the patients with recurrent urinary tract infections.

Previous studies on the functional damage following episodes of urinary tract infection have mostly been made in adult patients (12, 16, 17). Detailed descriptions of the radiological appearances of the patients are not given. Reduction of the GFR has been the predominant lesion. The glomerular tubular balance has been found to be preserved as far as the secretion of PAH (16, 17) and reabsorption of glucose (20) are concerned unless the glomerular filtration rate is reduced beyond 15 ml/min/1.73 m<sup>2</sup> b.s. The results have been interpreted to mean that in recurrent urinary tract infections there is a progressive complete destruction of the nephrons. The functional findings in children with recurrent urinary tract infections only partially agree with the findings in adults. In children functional changes can only be found if the IVP appearance is abnormal. The renal function can however not with any certainty be predicted by the radiological size of the kidney. Reduction of the GFR appears to be the dominant lesion also among children. Naturally this is partially due to an anatomical destruction of the nephrons. As has been suggested above a vasoconstriction related to changes in distal tubular sodium

reabsorption might contribute to the low GFR during hydropenia. The glomerular tubular balance as far as sodium is concerned appears to be reset at a higher level of glomerular filtration rate than what has been reported on the glomerular tubular balance for glucose. In agreement with Kleeman *et al.* (12) the patients with the most severe reductions of the GFR were unable to concentrate the urine maximally. In contrast however to what has been found in adults children with recurrent urinary tract infections appear to preserve their diluting capacity during water diuresis to a larger extent.

### SUMMARY

The glomerular filtration rate (GFR), urinary concentrating and diluting capacity and sodium excretion have been determined in 17 children with previous histories of urinary tract infection confirmed by urine cultures. There were no signs or symptoms of infection at the time of the study. Renal function was found to be normal in patients with normal intravenous pyelographies (IVPs). In the patients with renal parenchymal changes a wide variety of functional changes could be found. Reduction of the glomerular filtration rate was the predominant lesion. The GFR could however not be predicted by the IVP appearance. Most of the patients were able to increase the GFR during water diuresis. The glomerular tubular balance for sodium was reset in 3 of the patients as manifested by an increased  $C_{Na}/C_{Cr}$ . The concentrating capacity was also reduced in those 3 patients indicating an impairment of the sodium reabsorption in the loop of Henle. All the patients were able to dilute the urine normally during water diuresis but 5 of the patients were unable to increase their diluting capacity during the transition from hydropenia to water diuresis. It is noteworthy that only those patients were unable to increase the GFR significantly during the transition from hydropenia to water diuresis.

than the patients with normal IVPs. In contrast the group of patients with renal parenchymal changes who were unable to increase their diluting capacity during water diuresis, i.e. belonging to group IIb did not have any significant increase of the GFR.

The fractional sodium excretion increased slightly in all groups during the transition from hydropenia to water diuresis. This observation is in harmony with that made in healthy volunteers (3).

### DISCUSSION

The patients with normal IVPs demonstrate a wide variety of clinical histories. Two of the patients had the history of at most one infection, three of the patients had the history of one to three urinary tract infections yearly and one of the patients had had several episodes yearly of bacteriuria, high fever and unilateral abdominal pain radiating dorsally. Yet one of the most consistent findings of this study was the completely normal renal function found in those patients. The lack of significant difference in the results of children with recurrent urinary tract infections but with normal IVPs and the results of healthy adults is also of interest since it confirms the findings of other investigators that renal function after the age of 2 years appears to be fully developed and could well be related to body surface (8, 18, 21).

In all the patients with pathological appearance of the IVPs (group II) one or more of the parameters determined were impaired. Reduction of the GFR appeared to be the dominant change in renal function. Previous unilateral studies (4) have demonstrated that patients with recurrent urinary tract infections associated with renal parenchymal changes often demonstrate subclinical reductions of GFR. The unilateral studies also revealed that the filtration rate could not be simply related to the size of the renal parenchyma. The same lack of relationship could be demonstrated in 5 of the 11 patients in group II.

Three of the patients in group II exhibited

a marked increase in the fractional sodium excretion. All those patients had low filtration rates (67, 33 and 17 ml/min/1.73 m<sup>2</sup> b.s. respectively). It is well established that when the functioning population of nephrons is reduced either by ligation of branches from the renal artery (22) or by progressive renal disease (15) or by surgical removal (23) the urinary sodium excretion will increase. This reset of the level of glomerular tubular balance also demonstrated by the patients in this material is thought to be necessary for the control of fluid and sodium balance during declining renal function.

The finding of a reduced concentrating capacity indicates an impairment of the active sodium reabsorption in the loop of Henle, the main determinant of the counter current system (5). The concomitant finding of an increased fractional sodium excretion in those patients where the concentrating capacity was reduced supports this assumption. Since pyelonephritis is primarily a disease of the renal medulla, this localisation of functional damage could almost be predicted. A certain vulnerability of the active tubular sodium reabsorption during renal disease is also to be expected since this function is by far the most energy demanding process of the kidney (11, 13). It is thus somewhat remarkable that the impairment of the concentrating capacity, the dominant functional change during acute urinary tract infection (6, 14, 27, 28) is so readily reversible that it was only found in three of the patients studied.

The urinary diluting capacity as far as free water production is concerned was found to be normal in all the patients studied. Thus it can be expected that all the patients could adequately handle a given load of water. Yet if the results are analyzed more thoroughly it is found that five of the patients are unable to increase their diluting capacity in the distal tubule during the transition from hydropenia to water diuresis. This finding might have important implications for the control of renal hemodynamics in those patients. It is generally

## TREATMENT OF CYSTINOSIS WITH A DIET POOR IN CYSTINE AND METHIONINE

MOGENS FJORD CHRISTENSEN JOHN ALLAN NIELSEN and OLE HENRIKSEN

*From the Medical Department and Department of Chemical Chemistry  
Central Hospital Holstebro Denmark*

Cystinosis is an autosomal recessive inborn error of metabolism in which cystine because of still unknown mechanisms is accumulated in most organs and tissues giving rise to a de Toes-Debre-Fanconi syndrome photophobia and sometimes liver cirrhosis.

The disease has been extensively reviewed in several reports (2 4 5 10 14 16 17 18 22).

The classic treatment of cystinosis is symptomatic being directed against the rickets the hypopotassemia and the acidosis. Most often large doses of vitamin D and a solution of potassium and sodium citrate and citric acid are used. In spite of improvement during this therapy (3 12) the poor prognosis is not altered.

Penicillamine has been used as a substance which might reactivate the inactivated SH dependent enzymes and increase the excretion of cystine (2 6 9 15 16 18). Some authors are able to lower the high blood level of pyruvate and  $\alpha$ -ketoglutarate in their cystinotic patients but on the whole the results of such therapy have been unconvincing. Effective treatment with an anabolic steroid has been described in a single case (21).

As methionine is largely converted to cystine in the body it seemed obvious to try to reduce the storage of cystine with a diet poor in methionine (16). It seems that a diet which is

poor in both cystine and methionine (1 13 19) might achieve a better result even though this diet has been tried by some without objective evidence of any improvement (18).

Bauer & Antener (1) obtained a negative sulphur balance and improved both the tubular and glomerular function in a 2½ year old boy with a diet poor in cystine and methionine based on lentils powder. Moreover the treatment consisted of anabolic steroid choline chloride as a methyl donor vitamin D and periodic electrolytic supplement.

In the following report we will present our experiences in the treatment of 2 children with cystinosis. They were followed up for a year during treatment as proposed by Bauer & Antener (1) and the preliminary results have given us grounds for moderate optimism.

### CASE REPORTS

#### *Case 1*

**Family history.** L. R. M. a boy was the youngest child of four in a family with healthy but related parents (Fig. 1). The eldest child his sister is described below as case 2. His youngest brother died from severe dehydration when 2½ years old. An amino acid analysis on kidney tissue taken during the post mortem investigation showed storage of cystine in great quantities. His eldest brother is healthy.

**Case history.** The patient was born December 22nd 1965. Pregnancy and delivery were uncomplicated. His birth weight was 4000 g and his crown heel length

## ACKNOWLEDGEMENTS

The authors are indebted to Mrs Karin Egeld, Miss Liljebritt Jonsson and Miss Guye Svensson for expert technical assistance.

## REFERENCES

- 1 Aperia A C. *Studies on the regulation of renal hemodynamics*. Stockholm 1968.
- 2 — Tubular sodium reabsorption and the regulation of renal hemodynamics. The effect of chlorothiazide on renal vascular resistance. *Acta Physiol Scand* 75: 360 1969.
- 3 Aperia A C, Broberger O, Ericsson N O & Fejchting H. Renal function in man during transition from hydropenia to water diuresis with superimposed mannitol load. *Acta Physiol Scand* 77: 429 1969.
- 4 Berg U, Aperia A C, Broberger O, Ekengren A & Ericsson N O. Relationship between glomerular filtration rate and radiological appearance of the renal parenchyma in children. *Acta Paediatr Scand* 59: 1 1970.
- 5 Berliner R W & Bennett C M. Concentration of urine in the mammalian kidney. *Amer J Med* 42: 777 1967.
- 6 Brod J. Chronic pyelonephritis. *Lancet* 270: 973 1956.
- 7 Courtney M A, Nagel W & Thurnau K. A micropuncture study of the relationship between flow rate through the loop of Henle and sodium concentration in the early distal tubule. *Pflügers Archiv Ges Physiol* 287: 286 1966.
- 8 Edelmann C M Jr, Barnett H L, Stark H, Boychus H & Rodriguez Soriano J. A standardized test of renal concentrating capacity in children. *Amer J Dis Child* 144: 639 1967.
- 9 Goldberg M, McCurdy Donna K & Ramirez M A. Differences between saline and mannitol diuresis in hydropenic man. *J Clin Invest* 44: 182 1965.
- 10 Heyrowski A. A new method for the determination of inulin in plasma and urine. *Clin Chim Acta* 1: 470 1965.
- 11 Kuhl F, Aukland A & Refsum H E. Renal sodium transport and oxygen consumption. *Amer J Physiol* 201: 511 1961.
- 12 Kleeman C R, Hewitt W L & Guze L B. Pyelonephritis. *Medicine* 39: 3 1960.
- 13 Lassen N A, Munck O & Thaysen J H. Oxygen consumption and sodium reabsorption in the kidney. *Acta Physiol Scand* 51: 371 1961.
- 14 Longcope W T & Winklerwerder W L. Clinical features of the contracted kidney due to pyelonephritis. *Bull Hopkins Hosp* 53: 253 1933.
- 15 Lubowitz H, Purkerson M L & Bricker N S. Investigation of single nephrons in the chronically diseased (pyelonephritic) kidney of the rat using micropuncture techniques. *Nephron* 3: 73 1966.
- 16 Michie A J & Michie C R. Kidney function in unilateral pyelonephritis I. *Clinical data*. *Amer J Med* 22: 179 1957.
- 17 Michie A J, Michie C R & Rago M C. Kidney function in unilateral pyelonephritis II. Physiologic interpretations. *Amer J Med* 22: 190 1957.
- 18 Polacek E, Vogel J, Neugebauerova I, Sedlova M & Vecetlova E. The osmotic concentrating ability in healthy infants and children. *Arch Dis Child* 40: 291 1965.
- 19 Porush J D & Abramson R E. The effects of hypertonic mannitol on renal sodium excretion in hydropenic man. *Can Sci* 29: 475 1965.
- 20 Rieselbach R E, Shankel S W, Slatopolsky E, Lubowitz H & Bricker N S. Glucose permeation studies in patients with chronic progressive renal disease. *J Clin Invest* 46: 157 1967.
- 21 Rubin M J, Bruck E & Rapoport M. Alteration of renal function in childhood clearance studies. *J Clin Invest* 28: 1144 1949.
- 22 Schultze R O, Slatopolsky E, Tall B, Waller W, Levy M & Bricker N S. The regulation of sodium excretion in uremia: a new dimension in the characterization of third factor. *Ann Amer Phys Assoc* 79: 322 1966.
- 23 Schultze R O, Shapiro H S & Bricker N S. Studies on the control of sodium excretion in experimental uremia. *J Clin Invest* 48: 869 1969.
- 24 Smith H W. *The kidney. Structure and function in health and disease*. Oxford University Press New York 1951.
- 25 Thurnau K & Schnermann J. Die Natriumkonzentration an den Macula densa Zellen als regulierender Faktor für das Glomerulumfiltrat (Mikropunktionsversuche). *Klin Wochschr* 43: 410 1965.
- 26 Thurnau K. Renal hemodynamics. *Amer J Med* 36: 698 1964.
- 27 Winberg J. Renal concentration capacity during acute nonobstructive urinary tract infections in infancy and early childhood. *Acta Paediatr Scand* 47: 635 1958.
- 28 — Renal function studies in infants and children with acute nonobstructive urinary tract infections. *Acta Paediatr Scand* 48: 577 1959.

Submitted March 16 1970

Accepted March 20 1970

(A A) Dept of Paediatrics  
St Goran's Sjukhus  
Box 12 500  
112 81 Stockholm  
Sweden

Key words: Recurrent urinary tract infection; urinary concentrating capacity; urinary diluting capacity; renal tubular sodium reabsorption.

After that his mean daily intake of cystine and methionine did not exceed 25 mg and 90 mg per kg body weight respectively. This corresponds with the minimum methionine requirement and a much reduced content of cystine compared to the content in a normal diet.

During the modified dietary treatment he showed a striking improvement. The serum protein rose to normal values and the oedema and skin alterations disappeared. He was very soon in a good clinical condition with a good appetite. In March 1968 he began to speak with single words. In the beginning of April he could walk for a short while without support. He was kept in hospital until April 27th. Since then he has been admitted for a short period every third week. His general condition has continued to be good without relapses. By the termination of the follow up period he was able to run around, he was speaking in sentences and was as a rule gay and happy.

## Case 2

*Case history.* I. R. M. is an elder sister of the patient described above. She was born on June 25th 1960 as the first child of four. Delivery and pregnancy were normal. Her birth weight was 3500 g and her crown heel length 51 cm. She developed normally during the first 9 months of life but thereafter she began to vomit, she was constipated, refused to eat and failed to thrive. When 18 months old she was admitted to the paediatric department, Municipal Hospital Aalborg where a metabolic acidosis with lactic urine, constant proteinuria, hyperammonaemia and lowered serum phosphate were demonstrated. There were no clinical signs of rickets and no visible cystine crystals in the cornea. A renal tubular acidosis was suggested. A control admission was planned but the patient was not brought in. At the time of the admission to the Central Hospital in Aalborg her complaints were thirst and fatigue.

*Physical examination.* She was admitted on August 4th 1967. She presented as a dwarf with pronounced renal vulva due to severe rickets. Her height was 75 cm and weight 12.4 kg. She was pale with blue eyes, thin bloodless hair and remarkably chubby cheeks. She had photophobia and the slitlamp investigation showed cystine crystals in the cornea. She was easily tired, vomited now and then and had polyuria with nycturia 1-2 times each night. Her intelligence was normal. The results of the laboratory investigations on admission are shown in Table 1.

*Course.* She started the dietary treatment supplemented with cholecalciferol 500 mg each day and vitamin D<sub>3</sub> 0.5 mg each day on the 23th of August 1967. Besides this the classic treatment with vitamin D varying between 18 000 and 24 000 i.u. per day and periodically electrolyte supplements was given. From the very start of the treatment there was great difficulty in getting her to take sufficient amounts of the nourishment prepared from leek's meal.

She was kept in hospital until December 21st 1967

and thereafter she received attention as an inpatient for a few days every third week.

Because of increasing difficulty in giving her sufficient calories from the 5th of April the diet was supplemented with buttermilk in quantities varying from 250 to 500 ml per day. Thereafter her daily intake of methionine and cystine did not exceed 35 mg and 20 mg per kg body weight respectively.

The general condition was largely unchanged during the whole investigation period. She was easily tired and occasionally vomited. The serum protein was normal. The haemoglobin was constantly low, the anaemia being normochromic. On four occasions blood transfusions had to be given.

In spite of the above mentioned condition she started normal school in August 1968.

## METHODS

The effect of the treatment was estimated from the clinical condition and by repeated laboratory investigations including pyruvate and alaphetoxigenate in the blood, cystine and cystine excretion in the urine and amino acid determinations in urine and serum.

*Pyruvate and alaphetoxigenate.* For the analysis 200 µl of capillary blood treated with 200 µl of 3.5% perchloric acid were used. The analysis was performed immediately after the blood had been obtained with reagents from Boehringer (C. F. Boehringer and Soehne GMBH, Mannheim, Germany) and was transformed to a spectrophotometric method with a measuring volume of 160 µl. A spectrophotometer (Beckman DB) was used for the reading at 366 mµ. The normal values for adults are according to Boehringer 0.14-0.17 mg alaphetoxigenate and 0.4-0.6 mg pyruvate per 100 ml blood.

*Cystine and cystine excretion in the urine.* Urine was collected continuously during 24 hours in 5 ml 20 N sulphuric acid. It was kept cool during the collection. Cystine and cystine in the urine were precipitated as cystinecaptoproterceptate which was then after decomposition. On the addition of 2.6 dichloroethanechloride the liberated cystine formed a coloured compound that was measured in the spectrophotometer (8). The given values were calculated as cystine. The normal range for adults with this method is 8.2-18.1 mg per 24 hours (8).

*The amino acids in the urine.* were determined in the department of clinical chemistry, County Hospital in Aalborg with a Technicon amino acid analyzer. The normal values in Table 3 originate from the normal material of this laboratory of 29 persons in the age group of 3 to 25 years. For further description of the method and the material see Espersen & Peasey (7).

*The analysis of the serum amino acids.* was made in Biochromat, Copenhagen V on a Technicon amino acid analyzer with an elution time of 10 i. hours. The protein was precipitated with sulphosalicylic acid and after centrifugation the supernatant was applied to the column. The normal values in Table 4 are



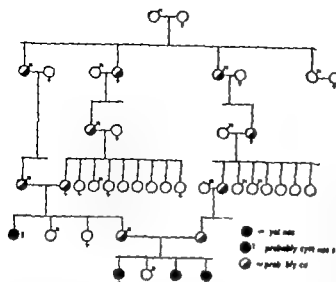


Fig 1 Pedigree. The father's oldest sister is indicated as a probable cystinosis patient. She died 2 years old after having shown a clinical picture much alike that of our patients.

was 54 cm. When 14 days old he had chicken pox but otherwise he was healthy and developed normally until the age of 7 months when his appetite became poor, he began vomiting, developed a remarkable thurst and failed to thrive.

**Physical examination.** He was admitted to the medical department Central Hospital in Holstebro on May 10th 1967. His weight was 8500 g and his height 70 cm. He was pale with blue eyes than blonde hair and with strikingly chubby cheeks. He had photophobia and examination with the slitlamp revealed cystine crystals in the cornea. Radiologically rickets was shown. He could sit up on his own and walk a little with support. His mental development was normal. A pronounced polyuria with a specific gravity of 1001-1002 was found and in spite of a fluid intake of about 5 litres per day he was occasionally dehydrated with flushing cheeks and a moderate fever. His appetite was bad and he often vomited. The results of the laboratory investigations on admission are shown in Table 1.

**Course.** Treatment was started with vitamin D from II to 45 000 international units (iu) per day according to the condition potassium citrate/sodium citrate solution corresponding to 25 mEq potassium and 25 mEq sodium per day, a combined vitamin preparation (Dokovit®) and oral iron. During this regime he continued to have a bad appetite, he often vomited and periodically he was slightly dehydrated.

From the 5th until the 28th of August the treatment was supplemented with penicillamine one capsule of 140 mg each day. Towards the end of this period he developed a severe vitamin D intoxication with serum calcium II 14.4 mg per 100 ml, frequent vomitings and a pronounced dehydration which demanded intravenous fluid and electrolyte supplements. Otherwise the general condition was unaltered.

The dietary treatment was started August 28th

1967. The diet is based on lentils meal as the protein source because this meal is poor in methionine and cystine. 100 g of the meal contains 155 g protein with 82-145 mg cystine and 116-146 mg methionine. Besides lentils meal nutrients poor in or free from protein were given. As a supplement to iron D cholinechloride 500 mg per day and an anabolic steroid metandienon (Dianabol®) 0.3 mg per day. The classic treatment was continued.

At the beginning of the dietary period he was in a quite good clinical condition but after 2 months he became increasingly debilitated with anorexia, vomiting and dehydration. He improved only partially after intravenous fluid and electrolyte supplement. There were increasing difficulties in giving him sufficient amounts of the diet and during the first fortnight of January 1968 he developed a state of malnutrition with low serum protein, the lowest value measured being 4.2 g per 100 ml. Simultaneously oedema of the lower extremities developed and the skin on the legs showed redness, infiltration and desquamation of large areas which were sharply outlined against the normal skin.

On the 14th of January 1968 we stopped the diet and gave cow's milk in free amounts. For 10 days the milk was enriched with 0.5% Neutradit hydrolysed animal protein containing free amino acids and with an amino acid nitrogen content of 6% of the preparation. From January 28th the diet was supplemented only with 500 ml cow's milk per day.

Table 1 Laboratory data at the admission

	Normal values	Case 1	Case 2
ESR (mm/hour)		11	5 <sup>2</sup>
Haemoglobin (g/100 ml)		10.1	9.1
Protein in serum (g/100 ml)	5.6-8.2	7.0	7.4
pH in capillary blood	7.35-7.43	7.30	7.31
Standard bicarbonate (mEq/l)	22.0-25.0	16.6	15.1
Chlorides in serum (mEq/l)	100-107	104	97
Sodium in serum (mEq/l)	136-148	134	133
Potassium in serum (mEq/l)	3.4-4.7	3.0	2.6
Calcium in serum (mg/100 ml)	9-11	10.0	8.1
Phosphorus in serum (mg/100 ml)	4.5-7.0	2.4	6.0
Alkaline phosphatase (k A units)	10-35	36.2	56.3
Creatinine in serum (mg/100 ml)	~1.2	0.9	2.3
Diuretics in ml		3620	1900
Protein in urine (g/l)		0.2	1.0
Glucose in urine (g/100 ml)		0.2	0.2
Potassium in urine (mEq/l)		10	21
Sodium in urine (mEq/l)		5	5 <sup>2</sup>
Calcium in urine (mg/24 hours)		0	82
Phosphate in urine (mg/24 hours)		320	220
pH in urine		6	7

Table 2 Pyruvate and  $\alpha$ -ketoglutarate in blood before and during dietary treatment<sup>a</sup><sup>a</sup> numbers of observations

	$\alpha$ -ketoglutarate (mg per 100 ml)			R P	Pyruvate (mg per 100 ml)			R P
	n	Mean	Range		n	Mean	Range	
Case 1								
Before treatment	8	0.908	0.30-0.78	35.5 $p < 0.01$	8	1.27	1.09-1.86	60 $p > 0.1$
During treatment	11	1.058	0.70-1.46		10	1.631	0.91-3.01	
Case 2								
Before treatment	4	0.417	0.34-0.45	8 $p < 0.01$	4	0.67	0.43-1.03	0.01 < $p$ < 0.02
During treatment	11	0.899	0.43-1.74		10	1.732	0.68-3.11	

For statistical analyses we used the Rank sum (Wilcoxon) test  
 Dornier's Geary Scientific Tables 6th ed pp 124-127

phosphate fluctuated between 3 and 4.4 mg per 100 ml. The serum creatinine remained normal. In case 2 slightly increasing metabolic acidosis, serum phosphate and serum creatinine were noticed, the serum creatinine being 3.3 mg per 100 ml at the end of the follow up period.

In case 1 the treatment had no influence on the glucosuria, the urinary pH or the diuresis. There was slight proteinuria on only a few occasions. The excretion of sodium, potassium and phosphate did not change during the dietary treatment. From the beginning of September 1967 calcium could be detected in the urine varying from 0 to 3.7 mg per 100 ml. In case 2 no change at all in the above mentioned values in the urine could be detected during the treatment.

$\alpha$ -Ketoglutarate and pyruvate levels in the blood during the treatment appear from Table 2. It is seen that the concentration of  $\alpha$ -ketoglutarate rose significantly in both patients. The blood concentration of pyruvate did not rise significantly in case 1 whereas a rise was highly probable in case 2.

The content of amino acids in urine and serum appears from Tables 3 and 4. The excretion of proline, citrulline, alanine and valine was pathologically increased in both patients. And the excretion of a number of the other

Table 3 Amino acids in urine ( $\mu$ moles per 24 hours)

	Normal values (range)	Lowest and highest values	
		Case 1	Case 2
Taurine	44-1303	0-23	0-217
Aspartic acid	8-158	6-171	22-88
Threonine	43-651	55-553	7-934
Serine	75-942	5-366	9-362
Glutamic acid	5-116	1-670	191-577
Proline	0	1250-6250	606-1793
Citrulline	0	116-537	118-248
Glycine	201-2780	793-1960	1129-1448
$\alpha$ -Alanine	67-799	475-2075	865-1409
$\alpha$ -Amino-iso-butyric acid	0	0	0
$\alpha$ -Amino-n-butyric acid	2-79	9-1562	15-56
Valine	10-12	132-557	163-820
Cystine	17-168	145-443	93-408
Methionine	3-39	0-9	17-94
Isoleucine	3-39	1-17	36-174
Leucine	6-164	9-32	33-397
Tyrosine	26-231	trace-438	16-213
Phenylalanine	15-168	trace-119	54-174
$\beta$ -Amino-iso-butyric acid	14-648	0-39	138-258
Ethionolamine	67-1168	0-107	0
$\gamma$ -Amino-n-butyric acid	0	0	0
Ornithine	3-92	0-135	56-134
Methylhistidine	33-966	0-trace	0-31
Lysine	28-482	465-1569	141-547
Tryptophane	9-100	0-68	28-33
Histidine	22-1671	367-622	153-422
Arginine	4-44	0-89	40-186

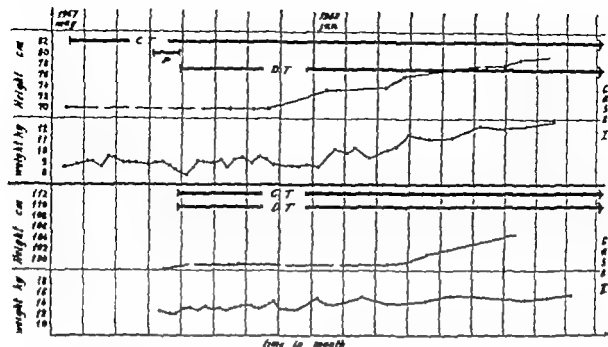


Fig 2 Height and weight in relation to the treatment  
CT classic treatment P penicillamine treatment  
DT dietary treatment

taken from investigations with the same equipment partly on 30 adults collected from 6 different investigations and partly on 20 children aging from 9 months to 2 years (20)

## RESULTS

**Classic treatment** of case 1 during the first 2½ months gave no improvement of the general condition and no gain in weight and height (Fig 2). The rickets was uninfluenced. The electrolyte state was only partly improved as metabolic acidosis, hypopotassemia, hyponatremia and hypercalcemia were occasionally present and serum phosphate was constantly below 4 mg per 100 ml. The great diuresis and the slight glucosuria were unchanged. The excretion of potassium in the urine varied from 10 to 20 mEq/l. The excretion of sodium in the urine was between 0 and 5 mEq/l until the middle of July 1967, after that time it was usually between 5 and 20 mEq/l. Calcium could not be detected in the urine during the first months and the urinary excretion of phosphate did not decrease.

**Penicillamine treatment** During the penicillamine treatment of case 1 there was no demonstrable improvement neither in the general

condition, the growth, the rickets, nor the electrolyte state. The only change was the hypercalcemic crisis at the end of the period.

We did not succeed in lowering the blood levels of alpha-ketoglutarate and pyruvate during the penicillamine treatment.

**Dietary treatment** As stated in the report there was no significant improvement of the general condition of case 1 until the child was supplemented with cow's milk. But from that time on there was a striking improvement with gain in weight and height (Fig 2). In case 2 the clinical development was not satisfactory even though there was a moderate gain in height (Fig 2).

The rickets healed in both cases during beginning of January 1968. The necessary doses of vitamin D were then tolerated without any hypercalcemia.

It was not possible to show quantitative changes of the cystine storage in the cornua during the treatment in either of the patients.

During the modified dietary treatment of case 1 a normalization of the serum acid-base status and sodium and calcium levels in the serum was obtained. But there were occasional slightly lowered potassium values and serum

in patients were not exceptions in this respect towards the end of 1967 when case 1 gradually took less of the lentils meal he went into clear state of malnutrition which might have been due either to a protein depletion or to a mix of amino acid imbalance (11) the very low content of cystine in the nourishment and then leading to falling appetite and food intake. A combination of both factors may have been responsible.

During the whole follow up period case 2 was rather reluctant to take the diet and tube feeding was considered on more than one occasion. On the modified diet both patients received more cystine, cysteine and methionine per day than advocated by Bauer & Antener (1) but since they turned best on that diet and as it implied a considerably reduced supply of sulphur containing amino acids we continued that treatment.

In the first patient the clinical result was good. It is impossible to decide whether this was due to the dietary treatment or to a late manifestation of the conservative treatment or to a combined effect of both treatments. We did not succeed in improving the kidney function or reducing the concentrations of pyruvate and  $\alpha$ -ketoglutarate with the treatment nor could a quantitative alteration in the cystine stores in the cornea be shown which means that there has been no demonstrable reduction in the cystine content of the body. Perhaps this could have been obtained on a more rigid diet. On the other hand there is no evidence of a progressive cystine storage during the treatment unless one will interpret the increased concentration in blood of  $\alpha$ -ketoglutarate as an indication of increased cystine storage.

In case 2 the results as could be expected were not so encouraging although the rickets could be healed and there was some gain in height. The treatment could not prevent an increasing glomerular kidney insufficiency and we can only suppose that the development of the uremia had been retarded.

The present work allows no ultimate judgment of the effectiveness of the dietary treat-

ment. However it seems as if manifest pathological changes will not be influenced by the diet.

The spontaneous course of this illness is so serious that any arrangement which will possibly reduce the cystine storage must be advisable. We must therefore advise adding to the conservative symptomatic treatment a dietary treatment which has to be started as early as possible in the course of the illness.

## SUMMARY

Two siblings with cystinosis are presented.

Case 1 a 16-month-old boy presented with a severe renal tubular insufficiency. Case 2 a 7 year-old girl was a dwarf with both glomerular and tubular renal insufficiency.

Case 1 was initially treated with high doses of vitamin D and electrolyte supplements for more than 2 months without significant alteration of the condition. Thereafter he was treated for 23 days with 150 mg penicillamine per day again without any significant clinical or biochemical improvement. Both patients were then followed through 1 year on treatment with a diet poor in cystine and methionine supplemented with cholecalciferol, an anabolic steroid, high doses of vitamin D, electrolytes, oral iron and a combined vitamin preparation. After some time there was considerable difficulty in giving the patients sufficient amounts of the diet, consequently the diet had to be modified with supplement of cow's milk. On this treatment case 1 attained a distinct clinical improvement with healed rickets and normal growth. There was no evidence of mobilization of the stored cystine.

Case 2 obtained a healing of the rickets and some gain in height during the treatment but otherwise the general condition was unaltered and she continued to have increasing renal glomerular insufficiency.

## ACKNOWLEDGEMENTS

The research foundation of the Central Hospital in Holstebro has partly supported this work. The lentils

Table 4 Amino acids in serum ( $\mu$ moles per l)

	Normal values (range)		Lowest and highest values	
	Adults	Children	Case 1	Case 2
Taurine	32-138	19-91	68-152	—
Aspartic acid	1-11	0-9	27-75	40-49
Threonine	76-194	33-128	45-168	7-239
Serine	76-164	24-172	84-155	7-152
Glutamic acid	20-90	20-90	131-259	157-179
Proline	103-290	51-185	92-427	278-355
Citrulline	—	—	8-56	56-90
Glycine	179-587	56-308	214-329	505-606
$\alpha$ -Alanine	213-472	99-313	264-587	408-557
$\alpha$ -Amino $\omega$ -butyric acid	10-35	0-17	23-30	12-15
Valine	168-317	57-262	56-333	116-196
Cystine	70-108	0-40	0-39	16-54
Methionine	11-30	3-29	6-43	16-31
Isoleucine	40-99	26-94	7-84	29-58
Leucine	78-176	43-155	32-168	62-118
Tyrosine	22-83	11-122	10-56	23-33
Phenylalanine	38-73	23-69	65-94	57-78
Ornithine	30-64	10-107	22-156	73-119
Lysine	105-207	45-144	53-280	134-213
Histidine	32-97	24-112	59-132	83-108
Arginine	40-140	11-65	61-144	125-158

amino acids was occasionally increased. The treatment did not normalize this condition.

The first investigation of serum amino acids in case 1 was done while he was hypoproteinaemic when the concentration of a number of amino acids was rather low. In the later investigations the concentration of several of the serum amino acids was a little higher than normal in both patients.

The excretion of cystine and cysteine in urine before and during dietary treatment is stated in Table 5. In case 1 it seems as if the concentration in the urine was lower during the treatment. In case 2 the concentration in the urine seemed greater during the treatment. The fluctuations from the normal adult range

Table 5 Cystine and cysteine in urine before and during dietary treatment

Case 1 mg/100 ml				
Before diet	4.8	4.8		
During diet	1.82	0.68	1.02	1.14
Case 2 mg/100 ml				
Before diet	0.94	0.42		
During diet	7.98	4.28	0.64	1.95

are not great, however, and the numbers of observations before the dietary treatment are too few to permit a statistical evaluation.

## DISCUSSION

As long as the aetiology of cystinosis remains unknown, uncertainty towards the proper treatment will exist. The uncertainty is augmented by the rarity of the disease giving great difficulty in gaining enough experience with new principles of treatment. The difficulties in estimating the value of new principles of treatment are in addition increased due to the rather different spontaneous course of the individual cases.

Bauer & Antener's (1) combined dietary medical treatment, however, seemed to be a great step forward that we chose to treat our 2 patients according to this principle.

It is not possible, on the basis of this investigation, to evaluate with certainty the effect of the classic treatment. It appears that case 1 was neither clinically nor biochemically improved during the more than 2 months of continuous classic treatment. Others (3, 12) during several years of continuous therapy with high doses of vitamin D and electrolyte supplement have obtained healed rickets and a normal or improved growth in their cystinosis patients.

The 23 days penicillamine treatment of case 1 did not alter his condition in a positive way. Even if penicillamine lowers the high blood concentration of pyruvate and  $\alpha$ -ketoglutarate in certain cystinosis patients, this is certainly not so in all cases (1) and some of the patients develop unpleasant side effects in connection with penicillamine treatment (1, 2). It is doubtful whether the hypercalcaemic crisis in our patient can be related to the penicillamine treatment.

There are often considerable difficulties in treating children with special diets. This appears very clearly when a cystinosis patient with anorexia is kept on the rigid and not very palatable cystine and methionine poor diet.

patients were not exceptions in this respect towards the end of 1967 when case 1 gradually took less of the lentils meal, he went into a clear state of malnutrition which might have been due either to a protein depletion or to a state of amino acid imbalance (11) the very low content of cystine in the nourishment and not leading to falling appetite and food intake. A combination of both factors may have been responsible.

During the whole follow up period case 2 was rather reluctant to take the diet and tube feeding was considered on more than one occasion. On the modified diet both patients received more cystine, cysteine and methionine than has been advocated by Bauer & Ammer (1) but since they thrived best on that diet and it implied a considerably reduced supply of sulphur-containing amino acids we continued that treatment.

In the first patient the clinical result was good. It is impossible to decide whether this was due to the dietary treatment or to a late manifestation of the conservative treatment or to a combined effect of both treatments. We did not succeed in improving the kidney function or reducing the concentrations of pyruvate and alpha-ketoglutarate with the treatment nor could a quantitative alteration in the cystine stores in the corners be shown which means that there has been no demonstrable reduction in the cystine content of the body. Perhaps this could have been obtained on a more rigid diet. On the other hand there is no evidence of a progressive cystine storage during the treatment unless one will interpret the increased concentration in blood of alpha-ketoglutarate as an indication of increased cystine storage.

In case 2 the results as could be expected were not so encouraging although the rickets could be healed and there was some gain in height. The treatment could not prevent an increasing glomerular kidney insufficiency and we can only suppose that the development of uremia had been retarded.

The present work allows no ultimate judgment of the effectiveness of the dietary treat-

ment. However it seems as if manifest pathological changes will not be influenced by the diet.

The spontaneous course of this illness is so serious that any arrangement which will possibly reduce the cystine storage must be advisable. We must therefore advise adding to the conservative symptomatic treatment a dietary treatment which has to be started as early as possible in the course of the illness.

## SUMMARY

Two siblings with cystinosis are presented.

Case 1 a 16-month-old boy presented with a severe renal tubular insufficiency. Case 2 a 7 year-old girl was a dwarf with both glomerular and tubular renal insufficiency.

Case 1 was initially treated with high doses of vitamin D and electrolyte supplements for more than 2 months without significant alteration of the condition. Thereafter he was treated for 23 days with 150 mg penicillamine per day again without any significant clinical or biochemical improvement. Both patients were then followed through 1 year on treatment with a diet poor in cystine and methionine supplemented with cholecalciferol, an anabolic steroid, high doses of vitamin D, electrolytes, oral iron and a combined vitamin preparation. After some time there was considerable difficulty in giving the patients sufficient amounts of the diet, consequently the diet had to be modified with supplement of cow's milk. On this treatment case 1 attained a distinct clinical improvement with healed rickets and normal growth. There was no evidence of mobilisation of the stored cystine.

Case 2 obtained a healing of the rickets and some gain in height during the treatment but otherwise the general condition was unaltered and she continued to have increasing renal glomerular insufficiency.

## ACKNOWLEDGEMENTS

The research foundation of the Central Hospital in Hølsbro has partly supported this work. The lentils

meal was kindly placed at our disposal by the Nestlé Company Dr Ilse Antener Vevey Switzerland is thanked for her great interest and valuable help in the dietary treatment

# REFERENCES

- 1 Bauer H & Antener I Eine wirksame diätetische und medikamentöse Cystinosebehandlung *Helv Paediat Acta* 21 19 1966
- 2 Berger H Antener I Brechbühler T & Stalder G Zum Problem der Cystinose Beobachtungen über den Cystinnachweis im Serum und therapeutische Erfahrungen mit Penicillamin und anabolem Hormon *Ann Paediat* 202 465 1964
- 3 Bickel H Die Entwicklung der biochemischen Läsion bei der Lignac-Fanconi'schen Krankheit *Helv Paediat Acta* 10 259 1955
- 4 Bickel H Baar H S Askey R Douglas A A Finch E Harris H Harvey C C Hickmans E M Philpott M G Smallwood W C Smellie J M & Teall C G Cystinose storage disease with aminoaciduria and dwarfism *Acta Paediat Scand Suppl* 90 1 1952
- 5 Brodehl J Hagge W & Gelsen K Die Veränderungen der Nierenfunktion bei der Cystinose *Ann Paediat* 205 131 1965
- 6 Chayton B E & Patrick A D Use of dimer kaprol or penicillamine in the treatment of cystinosis *Lancet* II 909 1961
- 7 Espersen O & Paaby P Ionbyttekromatografisk bestemmelse af aminosyrer i plasma og urin på et normalmateriale *Dan Med Bull* In press
- 8 Fernandez A A & Henry R J A highly specific new method for the determination of cystine and cysteine and its application to urine *Anal Biochem* 11 190 1965
- 9 Hambræus L & Broberger O Penicillamine treatment of cystinosis *Acta Paediat Scand* 56 243 1967
- 10 Hagge W & Brodehl J Die Veränderungen der Nierenfunktion bei der Cystinose *Ann Paediat* 205 442 1965
- 11 Harper A E Leung P Yoshida A & Rogers Q R Some new thoughts on amino acid im balance *Fed Proc* 23 1087 1964
- 12 Harrison H E The Fanconi syndrome *J Chron Dis* 7 346 1958
- 13 Linneweh F Möglichkeiten einer diätetischen Beeinflussung der Cystinose *Alm Wochr* 42 663 1964
- 14 Patrick A D Deficiencies of SH dependent enzymes in cystinosis *Clin Sci* 28 427 1965
- 15 Rosser A Caldera R & Odievre M Notion recentes sur la cystinose *Bull Soc Med Hop Paris* 114 1215 1963
- 16 Scharer K & Antener I Zur Biochemie und Therapie der Cystinose *Ann Paediat Suppl* 1 1 1964
- 17 Schneider J A Wong V Bradley K & Seegmüller J E Biochemical comparison of the adult and childhood forms of cystinosis *New Engl J Med* 279 1253 1968
- 18 Seegmüller J E Friedmann T Harrison H E Wong V & Schneider J A Cystinosis Combined Clinical Staff Conference at the National Institutes of Health *Ann Intern Med* 68 843 1968
- 19 Seip M Steen Johnsen J Vellan J E & Gjessing L R Dietary treatment of cystinosis *Acta Paediat Scand* 57 409 1968
- 20 Soupart P Free amino acids of blood and urine in the human in J T Holden (ed) *Amino acid pools* Elsevier Publishing Company Amsterdam London and New York 1962 p 220
- 21 Weber H & Hagge W Über die erfolgreiche Behandlung der Zystinose mit einem Anabolon *Arch Kinderheilk* 168 110 1963
- 22 Worthen H G & Good R A The de Toni Fanconi syndrome with cystinosis *Amer J Dis Child* 95 653 1958

Submitted September 18 1969

Accepted March 15 1970

(M F Chr)

Pediatric Department

Aarhus Kommunehospital

Aarhus C

Denmark

Key words Cystinosis penicillamine dietary treatment

## INTESTINAL DIPEPTIDASES AND DISACCHARIDASES IN CHILDREN WITH MALABSORPTION

A DAHLQVIST T LINDBERG G MEEUWISSE and M AKERMAN

*From the Department of Paediatrics Malmö General Hospital Malmö  
the Departments of Paediatrics and of Pathology and the Research Department I  
E Møller University Hospital Lund Sweden*

Celiac disease<sup>1</sup> is the most common protein induced malabsorption syndrome in childhood. We know that its clinical symptoms are precipitated by wheat gluten (9) but we do not know why. Indirect evidence suggests that the aetiological factor is a lack of a specific peptidolytic enzyme in the intestinal mucosa (5, 6, 13, 23, 25, 34, 35, 38). Using a recently developed method for quantitative assay of dipeptidase activities (dipeptidase hydrolases EC 3.4.13) in biopsy specimens of the small intestinal mucosa (19, 30) we have studied a number of infants and children with celiac disease before and after dietary treatment.

In the acute phase of celiac disease there is considerable mucosal damage leading to morphological alterations as well as unspecific decrease of for instance the disaccharidase activities. These changes are reversible at least partly on treatment with gluten free diet (1, 15, 18, 36, 40, 41, 43). Corresponding secondary changes could be expected to occur with the dipeptidase activities therefore the level of dipeptidase activities in the biopsy specimens studied was compared with the level of disaccharidase activities in the same specimens.

In a small group of our patients it appeared that the malabsorption was not gluten induced. These patients showed instead a clinical

course in many respects similar to that described in cow's milk induced intolerance (8, 14, 27, 44). Therefore these patients were put into a separate group here called "suspected cow's milk intolerance".

Some of the results have been presented in a preliminary form (29).

### MATERIAL

The patient material consists of 18 infants and children treated at the Paediatric Departments in Lund and Malmö from 1966 to 1969 for diarrhoea vomiting and/or failure to thrive.

One group includes 13 patients (6 boys and 7 girls) aged from 5 months to 13 years (Table 1). All showed a definite clinical improvement (i.e. their appetite became normal, stools normal and they gained weight) on gluten free diet (no wheat, rye, barley or oats) and their symptoms returned when given unrestricted gluten-containing diet.

"Suspected cow's milk intolerance" group includes 5 patients (3 boys and 2 girls) aged from 3 to 8 months (Table 2). They showed no or poor clinical improvement on gluten free diet and when by other dietary restrictions (omission of cow's milk) they became symptom free the administration of gluten did not cause any relapse. In four of them (14 J.J., 15 B.V.L., 17 M.L., 18 R.J.) cessation of cow's milk was tried and a marked clinical improvement ensued. Two of the four (14 J.J. and 18 R.J.) were given a provocation test with cow's milk that caused vomiting and diarrhoea. These 4 patients were fed human milk which they tolerated well. Between the ages of 8 and 15 months they began to tolerate cow's milk in small quantities. The fifth patient in the suspected cow's milk intolerance group (16 N.S.)

Also called: gluten induced enteropathy, celiac sprue, non-tropical sprue or idiopathic steatorrhea.



Table 1a Coeliac group Clinical and laboratory data (before diet)

Patient	Age (years)	Gluten free diet (months)	Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml)		Faecal fat (g/day)	Mucosal morphology	
					30 min	60 min		General view of villi	Histological score <sup>a</sup>
1 B B	1/2	0	-1.2	±0	15	25	— <sup>b</sup>	Absent	15
2 M J	1/2	0	-3.4	-1.8	(1 )		13	—	—
3 S N	3/4	0	-2.9	±0	(5 ) <sup>c</sup>		15	Convuluted	14
4 H L	3/4	0	-2.1	-1.4	16	24	1	Absent	17
5 J U	3/4	0	-2.4	-1.7	2	4	—	Absent	16
6 A L	3/4	0	-3.7	-1.2	—		12	—	—
7 M L	1	0	-2.8	-2.2	(10 )		11	Absent	17
8 Ch Z	1 1/4	0	-3.1	-3	24	30	5	Absent	18
9 V N	1 1/2	0	-2.5	-2.5	14	17	3	Absent	16
10 T E	1 3/4	0	-2.1	-2.4	14	19	5	Absent	17
11 S M	2	0	-2.3	-2.7	13	18	12	—	—
12 R S	7 1/2	0	-1	±0	18	27	4	Absent	18
13 L B P	13	0	-2.5	-2.5	2	3	37	Short broad	12
Normal values					~15	~25	<4		<4

<sup>a</sup> Histological score with respect to villous structure: changes of surface epithelium and infiltration with inflammatory cells: normal <4 points, slight damage = 9 points, grave damage >14 points.  
 — = not examined.

Urinary xylose excretion: normal values >15.

received gluten free diet containing cow's milk from age 6 to 25 months. During this period his condition improved very slowly but at 25 months his xylose absorption and his intestinal mucosa had become normal. From 27 months he tolerated an unrestricted diet and when his intestinal mucosa was studied after 9 months without any dietary restrictions it was found to be still normal.

Further data on some of the patients in this group have been presented (2).

**Control group.** Because of the difficulties involved in obtaining a strictly normal control material we have used a control group consisting of 12 infants and children (aged 2 months to 14 years) and 37 adults (3) with symptoms from the gastro-intestinal

Table 1b Coeliac group Clinical and laboratory data (after dietary treatment)

Patient	Age (years)	Gluten free diet (months)	Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml)		Faecal fat (g/day)	Mucosal morphology	
					30 min	60 min		General view of villi	Histological score <sup>a</sup>
1 B B	Not studied								
2 M J	3	29 <sup>d</sup>	+1.2	+1.8	15	16	2	Short broad	11
3 S N	2	16	+0.7	+0.1	36	88	3	Short broad	11
4 H L	Not studied								
5 J U	2 1/4	17	-0.2	-0.4	33	32	— <sup>b</sup>	Short broad	9
6 A L	2 1/2	0 <sup>c</sup>	-1.2	±0	29	42	7	Absent	17
7 M L	1 1/4	4	-1.7	-2.1	(12 )		1	Broad leaves	11
8 Ch Z	1 3/4	8	-0.7	-1.4	41	59	—	Finger leaves	7
9 V N	2	7	-0.5	-2.5	29	42	3	Convuluted	13
10 T E	2 1/2	10	-1.2	-2.0	28	16	4	Short broad	11
11 S M	3	12	-1.9	-2.6	41	43	2	Finger leaves	7
12 R S	8	9	-0.4	+1	40	49	2	Convuluted	14
13 L B P	14	9	-0.8	-1.6	22	50	—	Finger leaves	7
Normal values					~15	~25	4		<4

<sup>a</sup> For explanation see Table 1a.

<sup>b</sup> Not strictly gluten free diet all the time.

<sup>c</sup> Gluten free diet for 15 months, gluten-containing diet the last 4 months before biopsy.

The biopsy has therefore been included in the untreated group in Figs 1 and 2.

2a Suspected cow's milk intolerance group Clinical and laboratory data (before diet)

No.	Age (months)	Diet (months)		Body weight (S.D.)	Body height (S.D.)	Blood xylom (mg/100 ml)		Faecal fat (g/day)	Mucosal morphology	
		Cow's milk free	C'luten free			30 mm	60 mm		General view of villi	Histol score <sup>a</sup>
J	2	0	0	-2.3	-1.0	— <sup>b</sup>	—	7	—	—
M I	5	0	0	-2.9	-1.1	12	18	6	Convulsed	12
S	6	0	0	-2.3	-2.4	15	16	5	Absent	18
L	7	II	2	-2.2	-3.0	9	18	31	Absent	18
S	7	0	0	-1.0	±0	(10)	—	—	—	—
Med values						>13	>25	<4		<4

For explanation see Table 1a

1, but with histologically normal mucosa obtained biopsy. As there was no significant difference between values for enzyme activity in the children and in the adults the control material was combined into a single group.

## METHODS

### Intestinal tests

A possibility of pancreatic disease as a cause of lactose intolerance in our patients was excluded by determination of chymotrypsin in faeces (16) and resected. For determination of faecal fat excretion the child was fed a diet with known fat content and samples of the stools were analyzed for 3 days. The absorption of D xylom was studied after a meal of 15 g/m body weight. Blood xylom and/or urinary xylom excretion were determined (17, 12). Lactose and glucose-galactose tolerance tests were performed with a dose of 2 g/kg body weight.

### Biopsy technique

Biopsy of the small intestinal mucosa in all patients was taken at the duodeno-jejunal flexure under fluoroscopic control (We use a hydraulic multiple biopsy needle (Kirschner et al. to be published). We took 1-2 cm three specimens each weighing about 4 mg. One was used for morphological examination, one for assay of dipeptidase activity and one for disaccharidase activity. The pieces to be used for enzyme assay were enveloped airtight in paraffin and stored at -70°C until analysis. Stored thus the enzyme activities studied are stable for months.

### Mucosal histological examination

The biopsy specimen was oriented luminal side upwards on a fine plastic net and then fixed in Bouin solution or in formaldehyde solution. A short time after fixation in Bouin solution the fixed specimens were washed in dissection microscope and photographed. The same technician prepared the specimens and cut them serially in 5-6 µm sections taking care to keep

the cutting plane vertical to the mucosal surface. Alternative slides were stained with haematoxylin and erythrosin, with van Gieson stain and with PAS stain according to Kichians. The best orientated sections from the central part of the biopsy specimens were carefully studied in the light microscope and coded with respect to villous structure, changes of the surface epithelium and infiltration with plasma cells and neutrophil granulocytes (Berg et al.—in preparation) (See Table 1a).

### Assay of dipeptidase activities

The mucosal specimen was homogenized and assayed for dipeptidase activity as described earlier (19, 20). Glycyl-L-histidine (glycyl-L-histamine) was obtained from Cyclo Chem Co (Los Angeles Calif.). A 0.04 M aqueous solution was used. pH optimum was 7.7 (Lindberg unpublished). The other substrates were the same as used earlier (19, 20, 21). No metal ion activator was added (One unit of dipeptidase is the activity hydrolysing 1 µmole of dipeptide per min at 40°C).

### Assay of disaccharidase activities

After homogenization with a glass pestle the disaccharidase activities were analyzed by the method earlier described (7). (One unit of disaccharidase activity hydrolyses 1 µmole of disaccharide per min at 37°C).

### Assay of protein

The method of Lowry et al. (21) was used. In accord with earlier publications the disaccharidase activities were calculated as unit per g protein and the dipeptidase activities as units per mg nitrogen. The nitrogen content was calculated as 17% of the protein content.

The following abbreviations are used: ala glu (L-alanyl-L-glutamic acid), ala pro (L-alanyl-L-proline), gly leu (glycyl-L-leucine), gly val (glycyl-L-valine), glu pro (L-glutamyl-L-proline), glu val (L-glutamyl-L-valine) and val glu (L-valyl-L-glutamic acid).

Table 1a *Coeliac group Clinical and laboratory data (before diet)*

Patient	Age (years)	Gluten free diet (months)	Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml)		Faecal fat (g/day)	Mucosal morphology	
					30 min	60 min		General view of villi	Histological score <sup>a</sup>
1 B B	1/2	0	-12	±0	15	25	— <sup>b</sup>	Absent	15
2 M J	1/2	0	-34	-18	(1 ) <sup>c</sup>		13	—	—
3 S N	3/4	0	-29	±0	(5 )		15	Convuluted	14
4 H L	3/4	0	-21	-14	16	24	1	Absent	17
5 J U	3/4	0	-24	-17	2	4	—	Absent	16
6 A L	3/4	0	-37	-12	—		12	—	—
7 M L	1	0	-28	-22	(10 ) <sup>c</sup>		11	Absent	17
8 Ch Z	1 1/4	0	-31	-3	24	30	5	Absent	18
9 V N	1 1/2	0	-25	-25	14	17	3	Absent	16
10 T E	1 3/4	0	-21	-24	14	19	5	Absent	17
11 S M	2	0	-23	-27	13	18	12	—	—
12 R S	7 1/2	0	-1	±0	18	27	4	Absent	18
13 L B P	13	0	-25	-25	2	3	37	Short broad	12
Normal values					>15	~25	<4		<4

Histological score with respect to villous structure: changes of surface epithelium and infiltration with inflammatory cells: normal <4 points; slight damage = 9 points; grave damage >14 points.

— = not examined

Urinary xylose excretion: normal values >15

received gluten free diet containing cows milk from age 6 to 25 months. During this period his condition improved very slowly but at 25 months his xylose absorption and his intestinal mucosa had become normal. From 27 months he tolerated an unrestricted diet and when his intestinal mucosa was studied after 9 months without any dietary restrictions it was found to be still normal.

Further data on some of the patients in this group have been presented (2).

**Control group** Because of the difficulties involved in obtaining a strictly normal control material we have used a control group consisting of 12 infants and children (aged 2 months to 14 years) and 37 adults (3) with symptoms from the gastro-intestinal

Table 1b *Coeliac group Clinical and laboratory data (after dietary treatment)*

Patient	Age (years)	Gluten free diet (months)	Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml)		Faecal fat (g/day)	Mucosal morphology	
					30 min	60 min		General view of villi	Histological score <sup>a</sup>
1 B B	Not studied								
2 M J	3	29 <sup>d</sup>	+12	+18	15	16	2	Short broad	11
3 S N	2	16	+07	+01	36	88	3	Short broad	11
4 H L	Not studied								
5 J U	2 1/4	17	-02	-04	33	32	— <sup>b</sup>	Short broad	9
6 A L	2 1/2	0 <sup>e</sup>	-12	±0	29	42	7	Absent	17
7 M L	1 1/4	4	-17	-21	(12 )		1	Broad leaves	11
8 Ch Z	1 3/4	8	-07	-14	41	39	—	Finger leaves	7
9 V N	2	7	-05	-25	29	42	3	Convuluted	13
10 T E	2 1/2	10	-12	-20	28	16	4	Short broad	11
11 S M	3	12	-19	-26	41	45	2	Finger leaves	7
12 R S	8	9	-04	+1	40	49	2	Convuluted	14
13 L B P	14	9	-08	-16	22	50	—	Finger leaves	7
Normal values					15	~25	<4		<4

<sup>b</sup> \* For explanation, see Table 1a

<sup>c</sup> Not strictly gluten free diet all the time

<sup>d</sup> Gluten free diet for 15 months; gluten-containing diet the last 4 months before biopsy

The biopsy has therefore been included in the untreated group in Figs 1 and 2

Table 2a Suspected cow's milk intolerance group Clinical and laboratory data (before diet)

Patient	Age (months)	Diet (months)		Body weight (S.D.)	Body height (S.D.)	Blood xyllose (mg/100 ml)		Faecal fat (g/day)	Mucosal morphology	
		Cow's milk free	Gluten free			30 mm	60 mm		General view of villi	Histol score <sup>a</sup>
H.J.J.	2	0	0	-2.3	-1.0	— <sup>b</sup>	—	7	—	—
B.H.L.	5	0	0	-2.9	-1.2	12	18	6	Convulsed	12
M.N.S.	6	0	0	-2.3	-2.4	15	16	5	Absent	18
P.V.L.	7	0	2	-2.2	-3.0	9	18	24	Absent	18
B.R.I.	7	0	0	-1.0	±0	(10)	—	—	—	—
Secret xylase						<15	>25	<4	<4	

<sup>a</sup> For explanation see Table 1a

sect. but with histologically normal mucosa obtained as biopsy. As there was no significant difference between these 4 or enzyme activity in the child diet and in the adult, the control material was combined into a single group.

## METHODS

### Laboratory tests

The possibility of pancreatic disease as a cause of malabsorption in our patients was excluded by determination of chymotrypsin in faeces (16) and sweat test. For determination of faecal fat excretion the patient was fed a diet with known fat content and specimens of the stools were analyzed for 3 days (17). The absorption of D xyllose was studied after a dose of 15 g as body surface. Blood xyllose and/or urinary xyllose excretion were determined (37-32). Lactose and glucose-galactose tolerance tests were performed with a dose of 1 g/kg body weight.

### Biopsy technique

Biopsy of the small intestinal mucosa in all patients was taken at the duodeno-jejunal flexure under fluoroscopic control. (The use of a hydraulic multiple biopsy capsule. Merz et al. to be published). We took at least three specimens each weighing about 5 mg. One was used for morphological examination, one for assay of dipeptidase activity and one for an xylase assay was embedded aseptically in paraffin and stored at 20°C until analyzed. Stored thus the enzyme activities studied are stable for months.

### Histological examination

The biopsy specimen was oriented longitudinally upwards on a fine plastic net and then fixed in Bouin solution or in formaldehyde solution with short fixation in Bouin solution. The fixed specimen was studied on a dissection microscope and photographed. The tissue to be studied was prepared and cut three serially = 4-6-8 sections taking care to keep

the cutting plane vertical to the mucosal surface. Alternative slides were stained with haematoxylin and erythron with van Gieson stain and with PAS stain according to Michasov. The best orientated sections from the central part of the biopsy specimen were carefully studied in the light microscope and coded with respect to villous structure, changes of the surface epithelium and infiltration with plasma cells and neutrophil granulocytes (Berg et al. — in preparation) (See Table 1c).

### Assay of dipeptidase activities

The mucosal specimen was homogenized and assayed for dipeptidase activity as described earlier (19-20). Glycyl-L-glutamine (glycyl-L-glutamine) was obtained from Cyclo Chem Co (Los Angeles Calif.). A 0.04 M aqueous solution was used. pH optimum was 7.7 (Lindberg unpublished). The other substrates were the same as used earlier (19-20-21). No metal ion activator was added. (One unit of dipeptidase is the activity hydrolysing 1 µmole of dipeptide per min at 40°C).

### Assay of disaccharidase activities

After homogenization with a glass pestle the disaccharidase activities were analyzed by the method earlier described (7). (One unit of disaccharidase activity hydrolyses 1 µmole of disaccharide per min at 37°C).

### Assay of protein

The method of Lowry et al. (31) was used. In accord with earlier publications the disaccharidase activities were calculated as unit per g protein and the dipeptidase activities as units per mg nitrogen. The nitrogen content was calculated as 17% of the protein content.

The following abbreviations are used: al-glu (L-alanyl-L-glutamic acid), ala-pro (L-alanyl-L-proline), gly-leu (glycyl-L-leucine), gly-val (glycyl-L-valine), glu-pro (L-glutamyl-L-proline), glu-val (L-glutamyl-L-valine) and val-glu (L-valyl-L-glutamic acid).

Table 2b Suspected cow's milk intolerance group Clinical and laboratory data (after dietary treatment)

Patient	Age (years)	Diet (months)		Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml)		Faecal fat (g/day)	Mucosal morphology	
		Cow's milk free	Gluten free			30 min	60 min		General view of villi	Histol score <sup>a</sup>
14 J J	1/2	3	5	-0.9	-1.8	(20)	<sup>c</sup>	1	Finger leaves	7
15 B M L	1 1/4	0 <sup>a</sup>	10	-1.4	-1.3	10	18	—	Partly convoluted partly short broad	10
16 N M	2	0	19	-1.4	-2.3	33	47	—	Finger leaves	5
17 M L	1	0 <sup>a</sup>	6	-0.3	-0.6	11	21	—	Convoluted	11
18 R J	1 1/4	7	7	-0.2	-1.5	(10)		1	Finger leaves	9
Normal values						15	>25	<4		<4

<sup>a</sup> For explanation see Table 1a

<sup>b</sup> Cow's milk containing diet from 10 months of age

<sup>c</sup> Cow's milk containing diet from 6 months of age

Comments 14 Tolerating normal diet from 8 months 2 years observation 15 Tolerating normal diet from 1; 8 mo 2 years observation 16 Tolerating normal diet from 2 y 3 mo 1 year's observation 18 Tolerating normal diet from 1 y 3 mo 1 year's observation

## RESULTS

Some of the clinical and laboratory findings in the two patient groups are summarized in Tables 1 and 2. Tables 1a and 2a show the data before treatment and Tables 1b and 2b the data after. Body weight and height were calculated as standard deviations (S D) from the average of Swedish children (4-24).

Before dietary treatment xylose absorption was decreased in 9 of the 13 patients in the coeliac group (Table 1a) and in 4 of the 5 patients in the suspected cow's milk intolerance group (Table 2a). Faecal fat excretion was increased in 8 (out of 11 studied) in the coeliac group and in 4 of the patients in the other group. The morphological examination of the biopsy specimens revealed a flat mucosa of coeliac pattern (cf. Rubin & Dobbins (39)) in all the patients of the coeliac group and in 3 of those in the other group. In the two remaining patients of the suspected cow's milk intolerance group, biopsy was not performed before treatment.

After 4-29 months of gluten free diet treatment 10 of the patients in the coeliac group were again examined (see Table 1b). All had gained weight and showed improved xylose absorption. The faecal fat excretion was meas-

ured in 8 of them, it was normal in 7. The morphology of the intestinal mucosa showed a more or less marked improvement (Table 1b). Fig. 1 shows the dipeptidase activities in the group before and after treatment compared with the range of the control group and Fig. 2 the corresponding values for the disaccharidase activities. The enzyme activities showed a skewed distribution in a linear diagram, but a normal distribution when the corresponding logs were used (3). The enzyme activities were therefore plotted on a log scale. Before dietary treatment the dipeptidase activities in most of the coeliac patients were much lower than in the controls. The decrease was most pronounced for the  $\alpha_1$  pro dipeptidase activity. After treatment there was a marked increase in dipeptidase activity, thus in most cases values within or close to the range of controls were obtained. Also at this stage in relation to the coeliac group the  $\alpha_1$  pro dipeptidase activity was lower than in any of the other dipeptidase activities. None the less there was an increase also in the  $\alpha_1$  pro dipeptidase activity during treatment (Fig. 1). The  $\alpha_1$  val dipeptidase activity also behaved somewhat strangely, in 3 patients it decreased during treatment in the other it increased.

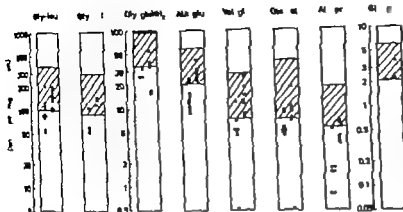


Fig 1 Dipeptidase activities in small intestinal biopsy specimens in the coeliac group ● Untreated ○ after treatment with gluten free diet ▨ controls range

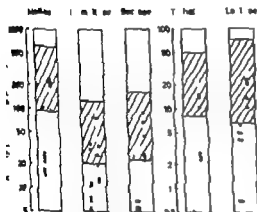


Fig 2 Disaccharidase activities in small intestinal biopsy specimens in the coeliac group For explanation of symbols see Fig 1

The disaccharidase activities were low at the first examination (Fig 2). The decrease in enzyme activity calculated as a percentage of the mean value for the control group was still more marked than for the dipeptidases (Table 3). The lactase and trehalase activities were proportionally somewhat more markedly decreased than the other disaccharidases. After treatment there was a considerable increase in all the disaccharidase activities (Fig 2 Table 3).

All 5 patients in the suspected cow's milk intolerance group were examined after 5-19 months of dietary treatment (Table 2b). All

Table 3 Specific activity of intestinal dipeptidases and disaccharidases in per cent of the mean values found in controls

Enzyme activity	Coeliac disease		Suspected cow's milk intolerance		Controls (n=49)
	Untreated (n=12)	Treated (n=10)	Untreated (n=3)	Treated (n=5) <sup>a</sup>	
Gly leu dipeptidase	45	76	33	64	100 (190)
Gly val dipeptidase	51	60	33	54	100 (172)
Gly glu+H dipeptidase	37	30			100 (62)
Ala glu dipeptidase	38	65	37	56	100 (36.3)
Val glu dipeptidase	31	87	49	52	100 (16.2)
Glu al dipeptidase	27	37	22	48	100 (18.9)
Ala pro dipeptidase	17	40	25	42	100 (10.8)
Glu pro dipeptidase	31	50	31	47	100 (3.6)
Maltase	16	30	12	50	100 (214)
Isomaltase	23	72	17	72	100 (57.2)
Sucrase	16	62	20	57	100 (60.2)
Trehalase	10	43	6	77	100 (22.1)
Lactase	10	40	3	46	100 (29.7)

Figures within parentheses are units/mg nitrogen (dipeptidases) and units/g protein (disaccharidases) respectively in the control group (mean value).

Table 2b "Suspected cow's milk intolerance" group Clinical and laboratory data (after dietary treatment)

Patient	Age (years)	Diet (months)		Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml)		Faecal fat (g/day)	Mucosal morphology	
		Cow's milk free	Gluten free			30 min	60 min		General view of villi	Histol score <sup>d</sup>
14 J J	1/2	5	5	-0.9	-1.8	(20)	7 <sup>c</sup>	1	Finger leaves	7
15 B M L	1 1/4	0 <sup>a</sup>	10	-1.4	-1.3	10	18	— <sup>b</sup>	Partly convoluted partly short broad	10
16 N S	2	0	19	-1.4	-2.5	35	47	—	Finger leaves	5
17 M L	1	0 <sup>a</sup>	6	-0.5	-0.6	11	21	—	Convoluted	11
18 R J	1 1/4	7	7	-0.2	-1.5	(10)	7 <sup>c</sup>	1	Finger leaves	9
Normal values						~15	~25	<4		<4

<sup>a, b, c</sup> For explanation see Table 1a<sup>a</sup> Cow's milk containing diet from 10 months of age<sup>b</sup> Cow's milk containing diet from 6 months of age<sup>c</sup> Comments: 14 Tolerating normal diet from 8 months 2 years observation 15 Tolerating normal diet from 1 y 8 mo 2 years observation 16 Tolerating normal diet from 2 y 3 mo 1 year's observation 18 Tolerating normal diet from 1 y 3 mo 1 year's observation

## RESULTS

Some of the clinical and laboratory findings in the two patient groups are summarized in Tables 1 and 2. Tables 1a and 2a show the data before treatment and Tables 1b and 2b the data after. Body weight and height were calculated as standard deviations (S D) from the average of Swedish children (4-24).

Before dietary treatment xylose absorption was decreased in 9 of the 13 patients in the coeliac group (Table 1a) and in 4 of the 5 patients in the suspected cow's milk intolerance group (Table 2a). Faecal fat excretion was increased in 8 (out of 11 studied) in the coeliac group and in 4 of the patients in the other group. The morphological examination of the biopsy specimens revealed a flat mucosa of coeliac pattern (cf Rubin & Dobbins (39)) in all the patients of the coeliac group and in 3 of those in the other group. In the two remaining patients of the suspected cow's milk intolerance group, biopsy was not performed before treatment.

After 4-29 months of gluten free diet treatment, 10 of the patients in the coeliac group were again examined (see Table 1b). All had gained weight and showed improved xylose absorption. The faecal fat excretion was meas-

ured in 8 of them, it was normal in 7. The morphology of the intestinal mucosa showed a more or less marked improvement (Table 1b). Fig. 1 shows the dipeptidase activities in this group before and after treatment compared with the range of the control group and Fig. 2 the corresponding values for the disaccharidase activities. The enzyme activities showed a skew distribution in a linear diagram, but a normal distribution when the corresponding logs were used (3). The enzyme activities were therefore plotted on a log scale. Before dietary treatment, the dipeptidase activities in most of the coeliac patients were much lower than in the controls. The decrease was most pronounced for the ala pro dipeptidase activity. After treatment, there was a marked increase in dipeptidase activity, thus in most cases values within or close to the range of controls were obtained. Also at this stage in relation to the control group the ala pro dipeptidase activity was lower than any of the other dipeptidase activities. None the less there was an increase also in the ala pro dipeptidase activity during treatment (Fig. 1). The glu val dipeptidase activity also behaved somewhat strangely, in 3 patients it decreased during treatment, in the other 7 it increased.

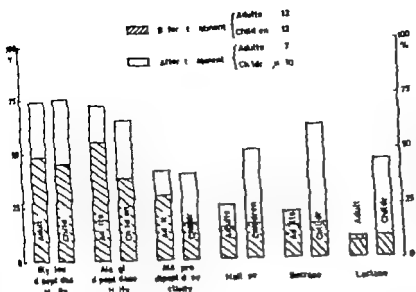


Fig. 5 Mean values of specific activities of intestinal dipeptidases and disaccharidases in coeliac disease in children and adults (3) expressed as per cent of the mean value for the corresponding activity in the control group

seem to be secondary in nature. The decrease was seen with all the dipeptidases and when calculated as percentages of the mean value of the control group the dipeptidase activities in our coeliac patients were not decreased to the same extent as were the disaccharidases (Table 3). The ala pro dipeptidase activity was apparently more strongly affected than the other dipeptidases with only 17% of the "normal" activity remaining before treatment. None the less four of the five disaccharidase activities studied were more strongly depressed than the ala pro dipeptidase. Furthermore during dietary treatment, the ala pro dipeptidase activity increased up to 40% of the "normal" value which would not be expected if this activity was the expression of an enzyme deficiency in coeliac disease. The glu val dipeptidase activity decreased in 3 cases during treatment but in the other 7 it increased parallel with the other enzyme activities. The unspecific decrease in the dipeptidase activities in the intestinal mucosa from patients with coeliac disease agrees with the results of several authors who have used different substrates and techniques (10, 12, 17, 35) but disagrees with the results of Messer et al. (33) who could not find any certain difference between the dipeptidase splitting capacity in untreated coeliac and normal

mucosa. To our knowledge 24 dipeptides have so far been tested by different authors in attempts to find a specific enzyme defect in coeliac disease.

There is a difference between the coeliac children studied here and coeliac adults (3) concerning the behaviour of the dipeptidases and the disaccharidases (Fig. 5). In adults and children the dipeptidase activities improved to the same level. The rise of the disaccharidases however was much slower in the adults than in the children. The mean lactase activity in the adults failed to improve. Both in children and adults the change in trehalase activity closely followed that of lactase although we know that these two activities are exerted by different enzymes.

The other patient group in our material is not so well defined as the coeliac group but we have good reasons for believing that those patients suffered from cow's milk intolerance. In some of them provocation tests with cow's milk were performed with positive result. Others were initially supposed to suffer from coeliac disease and were treated with gluten free diet, but after varying periods the diagnosis had to be revised and a diet with neither cow's milk nor gluten was introduced. The morphological changes in the mucosal biopsy



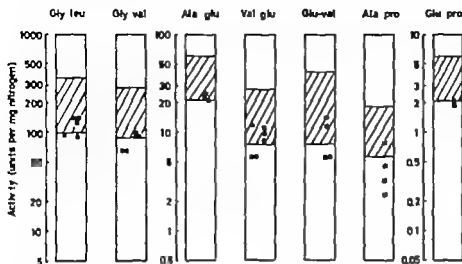


Fig 3 Dipeptidase activities in small intestinal biopsy specimens in the suspected cow's milk intolerance group ■ Untreated □ after dietary treatment, for details see text and Table 2 \* controls range

were given gluten free diet but cow's milk was excluded in only 2 of them. Furthermore only 3 of the patients had a biopsy taken before treatment, and although all 5 were biopsied after treatment, dipeptidase activities were measured in only 4 of them. Thus the data for this small group are rather incomplete. All gained weight during the dietary treatment, and the steatorrhea had disappeared, but in only two of them was the xylose absorption normal (Table 2b). Histologically the mucosa showed changes similar to those in the coeliac group and seemed to undergo a similar normalization during dietary treatment. The dipeptidase and disaccharidase activities were low and increased during dietary treatment

(Fig 3-4 Table 3). We could not find any morphological or enzymological differences between this group and the coeliac group. It has been reported that some patients with cow's milk intolerance develop a transient intolerance for gluten (14, 44) but this was not observed in our patients.

## DISCUSSION

All in our coeliac disease group seem to suffer from the classical form of the disease with a typical history, characteristic clinical and laboratory findings, and rapid improvement on gluten free diet. In 5 of our patients, we demonstrated a relapse on administration of gluten after the intestinal mucosa had first become normal (the criterion recommended by the European Society for Paediatric Gastroenterology for a strict diagnosis of coeliac disease). The others in this group have not yet been followed sufficiently long for this test to be performed, but all relapsed clinically when unrestricted diet was given after at least 6 months of gluten free diet.

The low disaccharidase activities found in the coeliac group and the increase in these activities on dietary treatment agree with earlier reports (1, 15, 18, 36, 40, 41, 43). These changes apparently occur parallel with the morphological changes in the mucosa.

The decreased dipeptidase activities in coeliac patients found with the substrates we used

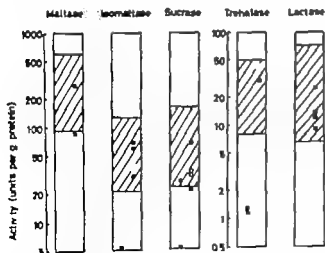


Fig 4 Disaccharidase activities in small intestinal biopsy specimens in the suspected cow's milk intolerance group. For explanation of symbols see Fig 3.

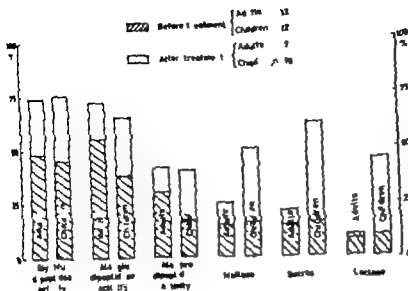


Fig. 5 Mean values of specific activities of intestinal dipeptidases and disaccharidases in coeliac disease in children and adults (3) expressed as per cent of the mean value for the corresponding activity in the control group.

seem to be secondary in nature. The decrease was seen with all the dipeptidases and when calculated as percentages of the mean value of the control group the dipeptidase activities in our coeliac patients were not decreased to the same extent as were the disaccharidases (Table 3). The *ala pro* dipeptidase activity was apparently more strongly affected than the other dipeptidases with only 17% of the "normal" activity remaining before treatment. None the less four of the five disaccharidase activities studied were more strongly depressed than the *ala pro* dipeptidase. Furthermore during dietary treatment, the *ala pro* dipeptidase activity increased up to 40% of the "normal" value which would not be expected if this activity was the expression of an enzyme deficiency in coeliac disease. The *glu val* dipeptidase activity decreased in 3 cases during treatment but in the other 7 it increased parallel with the other enzyme activities. The unspecific decrease in the dipeptidase activities in the intestinal mucosa from patients with coeliac disease agrees with the results of several authors who have used different substrates and techniques (10, 12, 17, 35) but disagrees with the results of Meiser et al. (33) who could not find any certain difference between the dipeptide splitting capacity in untreated coeliac and normal

mucosa. To our knowledge 24 dipeptides have so far been tested by different authors in attempts to find a specific enzyme defect in coeliac disease.

There is a difference between the coeliac children studied here and coeliac adults (3) concerning the behaviour of the dipeptidases and the disaccharidases (Fig. 5). In adults and children the dipeptidase activities improved to the same level. The rise of the disaccharidases however was much slower in the adults than in the children. The mean lactase activity in the adults failed to improve. Both in children and adults the change in maltase activity closely followed that of lactase although we know that these two activities are exerted by different enzymes.

The other patient group in our material is not so well defined as the coeliac group but we have good reasons for believing that these patients suffered from cow's milk intolerance. In some of them provocation tests with cow's milk were performed with positive result. Others were initially supposed to suffer from coeliac disease and were treated with gluten free diet, but after varying periods the diagnoses had to be revised and a diet with neither cow's milk nor gluten was introduced. The morphological changes in the mucosal biopsy

from these patients were very similar to those in the coeliac group. The only other condition with such changes recognized in Scandinavian paediatric practice and affecting the proximal part of the gut is cows milk intolerance (26-44). This intolerance disappeared at about one year of age in our patients which agrees with earlier reports (14-44).

In both coeliac disease and cows milk intolerance, damage of the intestinal mucosa seems to be provoked by a dietary protein. Thus for the cows milk intolerance, as well as for the coeliac disease, it is possible that the absence of some enzyme necessary for protein or peptide hydrolysis is responsible for the disease. Nor in this group however did our dipeptide substrates provide any clue to the specificity of such a hypothetical enzyme. The dipeptidase and disaccharidase activities tested were all low in the untreated phase of the disease. The findings of the disaccharidases agree with those reported by Silver & Douglas (42). This decrease of enzyme activity was reversible on dietary treatment. In fact, the similarity in the changes in the mucosal enzyme activities in the suspected cows milk intolerance group to those in the coeliac group was as striking as the morphological changes in the two groups.

### SUMMARY

Intestinal enzyme activities (dipeptidases and disaccharidases) and mucosal morphology were studied in two groups of paediatric patients: one coeliac group ( $n=13$ ) and one suspected cows milk intolerance group ( $n=5$ ). The patients were studied before and after dietary treatment. 49 children and adults with histologically normal intestinal mucosa served as a control group.

Both groups of patients showed a marked secondary decrease in the dipeptidase and the disaccharidase activities. The decrease was more pronounced for the disaccharidase than for the dipeptidase activities and was reversible on dietary treatment. Among the disaccharidases the lactase and trehalase activities

were most strongly depressed, in the coeliac group the ala pro dipeptidase activity was more strongly affected than the other dipeptidases. However, as also the ala pro dipeptidase activity increased on dietary treatment, this does not provide any clue to the specificity of a hypothetical enzyme whose absence could be responsible for the disease.

### ACKNOWLEDGEMENT

This work was supported by grants from the Swedish Medical Research Council (Projects B70 13P 276, B70 13X 157 and B68 61P 2359). The University of Lund, Semper Nutrition Foundation, the Swedish Nutrition Foundation, A. Pålsson's Foundation and A. Robert's Foundation.

The authors gratefully acknowledge the excellent technical assistance of Mrs A. Andersson, Mrs A. M. Andersson, Mrs C. Callmar, Miss M. B. Dahl, Mr L. Hansson, Miss U. Isarsson and Mrs B. Noren.

### REFERENCES

1. Arthur A. B. Intestinal disaccharidase deficiency in children with coeliac disease. *Arch Dis Child* 41: 519 1966.
2. Berg N. O., Dahlqvist A., Lindberg T., Mies Wasse G. W. & Ålerman M. Small intestinal mucosal structure, disaccharidases and dipeptidases in different types of malabsorption in childhood. A longitudinal study. International Conference on Coeliac Disease, London 1969.
3. Berg N. O., Dahlqvist A., Lindberg T. & Norden A. Intestinal dipeptidases and disaccharidases in coeliac disease in adults. *Gastroenterologia* In press 1970.
4. Broman B., Dahlberg G. & Lichtensten A. Height and weight during growth. *Acta Paediat Scand* 30: 1 1942.
5. Bronstein H. D., Haefliger L. J. & Kowleser O. D. Enzymatic digestion of gluten. The effect of the resultant peptides in adult coeliac disease. *Clin Chim Acta* 14: 141 1966.
6. Crabbe P. Sprue non tropical. Étiologie et pathogénie. *Acta Gastroent Belg* 27: 8 1964.
7. Dahlqvist A. Assay of intestinal disaccharidases. *Anal Biochem* 22: 99 1967.
8. Davidson M., Burnstone R. C., Kugler M. M. & Bruer C. H. Malabsorption defect induced by ingestion of beta lactoglobulin. *J Pediatr* 66: 545 1965.
9. Dickle W. K., Weijers H. A. & Lamer J. H. van de Coeliac disease II. The presence in wheat of a factor having a deleterious effect in cases of coeliac disease. *Acta Paediat Scand* 42: 34 1953.
10. Dolly J. O. & Fottrell P. F. Multiple forms of dipeptidases in normal human intestinal mucosa.

- and in mucus from children with coeliac disease  
*Clin Chim Acta* 26 555 1969
- 11 Douglas A P & Booth C C Jejunal mucosal  
deposition of gluten peptides in adult coeliac dis-  
ease *Lancet* 2 491 1968
- 12 Douglas A P & Peters T J Studies on intes-  
tinal peptidases with special reference to coeliac  
disease International Conference on Coeliac  
Disease London 1969
- 13 Frazer A G Discussion on some problems of  
steatorrhea and reduced stature *Proc Roy Soc  
Med* 49 1009 1956
- 14 Füllström S P Wisberg J & Andersen H J  
Cow's milk induced malabsorption as a precursor  
of gluten intolerance *Acta Paediatr Scand* 54  
101 1965
- 15 Hampton J F Lynch M J & Reilly B J  
Active coeliac disease in childhood Chemical and  
laboratory findings of forty-two cases *Quart J  
Med* 31 135 1969
- 16 Haverback B J Dyes B J Gotschall P J &  
Montgomery II W Measurement of trypsin  
and chymotrypsin in stool A diagnostic test for  
pancreatic exocrine insufficiency *Gastroenterology*  
44 588 1963
- 17 Heizer W D & Laster L Peptide hydrolase  
activities of the mucosa of human small intestine  
*J Clin Invest* 48 210 1969
- 18 Jon J Frézal J Rey J & Lamy M Étude  
histochimique de la mucosité duodéno-jéjunale  
dans la maladie coéliquée *Pediatr Res* 1 27  
1967
- 19 Josefsson L & Lundberg T Intestinal dipeptidase  
I Spectrophotometric determination and char-  
acterization of dipeptidase activity in pig intes-  
tinal mucosa *Biochim Biophys Acta* 105 149  
1963
- 20 — Intestinal dipeptidases III Characterization  
and determination of dipeptidase activity in adult  
rat intestinal mucosa *Acta Physiol Scand* 66  
410 1966
- 21 — Intestinal dipeptidases IX Studies on dipep-  
tidases of human intestinal mucosa *Acta Chem  
Scand* 21 1965 1967
- 22 Jansen van de J H van Bokkelt-Huysink H &  
Weyers H A Rapid method for the determina-  
tion of IgA in the faeces *J Biol Chem* 177 347  
1949
- 23 Jansen van de J H & Weyers H A The diag-  
nosis of protein malabsorption Proceedings Eu-  
rop Soc Paediatr Gastroenterol Interlaken 1969
- 24 Karlberg P Engström I Lohrström H &  
Svanberg I The development of children in a  
Swedish urban community A prospective longi-  
tudinal study III Physical growth during the  
first three years of life *Acta Paediatr Scand*  
Suppl 187 48 1968
- 25 Kratz I H G & Mohr H Weitere Unter-  
suchungen über den schädlichen Weizenmehl-  
fehl bei der Cöliakie 2 Die Wirkung der ent-  
sprechenden Arbeitsprodukte des Glutens *Helv  
Paediatr Acta* 14 1-4 1959
- 26 Kristman P Vinkorp J & Hallman N  
Histopathology of duodenal mucosa in malabsorp-  
tion syndrome induced by cow's milk *Ann Pa-  
ediatr* 205 54 1965
- 27 Lamy M Nezziol C Jos J Frézal J &  
Rey J La toposse de la muqueuse intestinale  
dans des syndromes de malabsorption *Presse  
Med* 71 1267 1963
- 28 Lundberg T Intestinal dipeptidases Dipeptidase  
activity in the mucosa of the gastrointestinal tract  
of the adult human *Acta Physiol Scand* 66  
437 1966
- 29 — Dipeptidase activity in small intestinal mu-  
cosa in patients with gluten induced enteropathy  
*Acta Paediatr Scand* Suppl 177 23 1967
- 30 Lundberg T Nordén A & Josefsson L In-  
testinal dipeptidases Dipeptidase activities in  
small intestinal biopsy specimens from a clinical  
material *Scand J Gastroint* 3 177 1968
- 31 Lowry O H Rosenbrough N I Farr A L  
& Randall R J Protein measurement with the  
Folin phenol reagent *J Biol Chem* 193 262  
1951
- 32 Mecturien G W & Dano G The xylase ab-  
sorption test in infancy *Acta Paediatr Scand* 54  
33 1965
- 33 Møller M Anderson Ch M & Townley R R  
W Peptidase activity of biopsies of the duodenal  
mucosa of children with and without coeliac  
disease *Clin Chim Acta* 6 768 1961
- 34 Møller M Anderson Ch M & Hubbard L  
Studies on the mechanism of destruction of the  
toxic action of wheat gluten in coeliac disease by  
crude pepsin *Gut* 5 295 1964
- 35 Pittman F E & Pollitt R J Studies of jejunal  
mucosal digestion of peptic tryptic digesta of  
wheat protein in coeliac disease *Gut* 7 368  
1966
- 36 Plotkin G R & Iselbacher A J Secondary  
disaccharidase deficiency in adult coeliac disease  
(non tropical sprue) and other malabsorption  
states *New Eng J Med* 271 1033 1964
- 37 Roe E W & Rice J H A photometric meth-  
od for the determination of free pantoic acid  
in animal tissues *J Biol Chem* 173 507 1948
- 38 Roon J H van & Haer A J Ch Clinical and  
biochemical analysis of gluten toxicity Mo II  
*Gastroenterologie* 94 2-7 1960
- 39 Rubin C E & Dobbas W O III Peroral  
biopsy of the small intestine A review of its  
diagnostic usefulness *Gastroenterology* 49 676  
1965
- 40 Scherlag D H Avricchio S Rabano A,  
Haden B & Prader A Der sekundäre Mangel  
an intestinaler Disaccharidaseaktivität bei der  
Coeliakie Quantitative Bestimmung der Enzyme  
aktivität und klinische Beurteilung *Helv Pa-  
ediatr Acta* 19 507 1964
- 41 Sheldon W & Tempney E Small intestine per-  
oral biopsy in coeliac children *Gut* 7 481  
1966

- 42 Silver H & Douglas D M Milk intolerance in infancy *Arch Dis Child* 43 17 1968
- 43 Welsh J E Zschecho O M Anderson J & Walker A Intestinal disaccharidase activity in celiac sprue (gluten sensitive enteropathy) *Arch Intern Med* 123 33 1969
- 44 Visakorpi J A & Immonen P Intolerance to cows milk and wheat gluten in the primary malabsorption syndrome in infancy *Acta Paediat Scand* 56 49 1967

Submitted April 6 1970

Accepted April 24 1970

(T L) Dept of Paediatrics  
Malmö Allmänna Sjukhus  
214 01 Malmö  
Sweden

**Key words** Celiac disease cows milk intolerance  
intestinal dipeptidases intestinal disaccharidases

## THE LOWE SYNDROME

### *Observations on the Amino Acid Metabolism in a 2 year-old Affected Boy*

LEIF HAMBRAEUS, GUNNAR PALLISCAARD and POUL MILDEBERG

*From the Perinatal Research Laboratory University Hospital Uppsala Sweden and  
the Department of Paediatrics Odense University Hospital Odense Denmark*

Since the original description by Lowe et al (9) in 1952 a substantial number of reports on the oculo-cerebro-renal syndrome have been published and an extensive review of the clinical and laboratory findings in this disease based on 57 cases from the literature was recently offered by Abbasi et al (1). In despite of the fact that marked aminoaciduria is one of the salient characteristics of the disease rather few quantitative studies of amino acid concentrations in blood, urine and cerebrospinal fluid are available.

The present report concerns a 2 year-old boy in whom a diagnosis of oculo-cerebro-renal syndrome was made at the age of 7 months when he was admitted to the Pediatric University Clinic in Odense. Studies of the amino acid pattern of blood, urine and cerebrospinal fluid by means of ion exchange chromatography showed the aminoaciduria to be of a fairly selective type.

## METHODS

Specimens of blood, urine and cerebrospinal fluid were sent from Odense to the Perinatal Research Laboratory in Uppsala and deproteinized within 36 hours of the time of sampling. Deproteinization was accomplished by adding 100 mg/ml solid sulphosalicylic acid.

This investigation was supported by grants from the Swedish Medical Research Council (B 49 1970, 767 04A) from the Tri-Centennial Fund of the Bank of Sweden and from Gertruds och Ivar Falckens Stiftelse University of Uppsala.

to each 2 ml sample of serum or cerebrospinal fluid. Following thorough mixing the samples were centrifuged the supernatant being stored at  $-20^{\circ}\text{C}$  until processed. Urine specimens were stored at the same temperature.

Amino acid concentrations were determined by means of either of two automatic amino acid analyzers one built at the Laboratory to the specifications of Spackman et al (16) and one commercial (Biochemical BC 200). Both apparatuses permitted analysis to be carried out on 0.2 ml of deproteinized serum and on 1.0 ml of deproteinized cerebrospinal fluid.

## Case Report

A C male infant born on May 26th 1967. He was the third child of healthy non-related parents. The parents first child was a stillborn boy the second a healthy now 7 year-old girl. An investigation of the family disclosed five additional cases of Lowe's syndrome the subject of another report (12).

The patient was born at term after a pregnancy uneventful except for the occurrence of mild transitory vaginal bleeding in the fourth month. Delivery was uncomplicated birth weight 3600 g and length (CRL) 36 cm. The testes were not descended. Shortly following birth ocular clouding as well as marked muscular hypotonia were noted and at the age of 11 days an ophthalmological examination confirmed the presence of bilateral cataracts.

At the age of 23 days the boy was admitted to the Pediatric University Clinic in Odense where he is still (March 1970) an inpatient. His psycho-motor development has been severely retarded and since the age of 3 months his weight has been below the 25 percentile. The muscular hypotonia noted at birth is associated with hyperflexibility of the joints and with absence of tendon reflexes. He is now almost 3 years old and weighs 9 kg. The length is 78 cm head circumference 52 cm chest circumference 46 cm. He is blind and profoundly oligophrenic (Fig 1).

In addition to the cataract severe bilateral glau-



Fig 1 The patient one year old

coma with enlargement and progressive clouding of the corneas and with very high pressures developed during the first year of life and several unsuccessful attempts at medical or surgical alleviation of the ocular hypertension including goniotomies and eversion of the right anterior camera were made.

Intravenous urographies disclosed a left distal ureteral structure with marked dilatation of the left urinary tract. At the age of 7 months surgical procedures including plastic operations on the left ostium and on the vesical culum were performed. Urinary tract infections have been treated early and the serum concentrations of creatinine and urea remain normal.

The presence of renal rickets was demonstrated at the age of 7 months when treatment with vitamin D was initiated. The occurrence of rachitic bone changes was preceded by the development of a metabolic acidosis. A blood acid base study at the age of 4 weeks was within normal limits although the blood titratable base whole blood base excess (BE) determined according to Astrup et al (2) was on the negative side  $-3.7$  mEq/l. Three months later the patient was definitely acidotic the BE being  $-11.5$  mEq/l (standard bicarbonate  $15.3$  mEq/l). From the age of 10 months the patient was given a citrate buffer (approx.  $1000$  mEq of potential base per liter) administered orally in doses of 6 to 8 mEq

per kg body weight per day. A survey of the large number of acid-base studies performed throughout the course reveals a striking lack of effect of this treatment. Spontaneous fluctuations of the blood BE level (limits zero and  $-11$  mEq/l) occurred apparently unrelated to the actual level of base administration. At the age of 27 months when the patient was receiving 50 mEq of citrate a day and had a blood BE of approximately  $-8$  mEq/l the rate of renal net acid excretion (7) was  $+3.6 \pm 0.6$  and  $-5.0$  mEq per 24 hours on three consecutive days. When evaluated in terms of the actual level of blood BE using the data of Kildeberg (8) and an estimated body surface area of  $0.41$  m<sup>2</sup> these values are pathologically low. On other occasions even more negative values (approx.  $-20$  mEq per 24 hours) were obtained. Urine pH values as low as 5 were found following temporary withdrawal of citrate therapy and the rate of renal ammonia production was invariably higher than that of the excretion of titratable acid.

Thus the presence of renal acidosis is well established. Furthermore the persistently high serum chloride levels ( $105$ – $120$  mmol/l) the normal serum concentrations of creatinine and of urea as well as the development of rickets serve to characterize the disturbance as a renal tubular acidosis. In our case neither deficient renal ammonia excretion nor failure to produce a normal plasma/urine pH gradient can be reasonably cited as causes of the metabolic acidosis. Rather the failure of base administration in doses equivalent to more than five times the expected rate of endogenous production of acid about  $1$  mEq per kg per day (8) to correct the acidosis and renal losses of base in the presence of renal tubular acidosis suggest a low renal bicarbonate threshold to be instrumental in the maintenance of the acidotic state.

#### Additional routine studies

Routine studies included normal electrocardiograms and normal electroencephalograms. X-ray studies of the skeleton including the skull revealed nothing abnormal except for retarded bone age (1<sup>st</sup> months at the chronological age of 21 months) and rachitic changes subsiding during the second year of life. A chromosomal analysis (Dr J. Mohr and Dr J. Schetz Larsen, Copenhagen) showed a normal pattern of chromosomes.

The blood hemoglobin concentration varied between  $10.0$  and  $13.1$  g per 100 ml the serum creatinine concentration between  $0.5$  and  $0.6$  mg per 100 ml. A study of the proteins in serum at the age of 11 months gave the following results: total concentration  $9.4$  g per 100 ml (albumin  $48\%$ , globulin  $3\%$ ,  $\alpha_1$  globulin  $20\%$ ,  $\beta$  globulin  $12\%$ ,  $\gamma$  globulin  $17\%$ ). Immunoelectrophoresis of the proteins in serum showed increased concentrations of IgA, IgG and IgM (Dr J. Clausen, Copenhagen). The serum concentration of  $\beta$  microglobulin was found to be  $2.5$   $\mu$ g per ml (Dr P. E. Ervin, Uppsala).

During the first 3 months of life intermittent

glutamine was present. Proteinuria has been a constant finding concentrations varying between 0.9 and 2.5 g/l. An electrophoretic study of the urinary protein at the age of 11 months gave the following results: total concentration 2.2 g/l (albumin 32%,  $\alpha$ -globulin 10%,  $\alpha_2$ -globulin 31%,  $\beta$ -globulin 16%,  $\gamma$ -globulin 21%). At the age of 21 months the rate of urinary loss of  $\beta$ -microglobulin was 57 mg per 24 hours.

### Amino acids

The results of quantitative amino acid analyses on specimens of serum or urine obtained on two different occasions are presented in Tables 1, 2, and 3. The data of Table 1 show that the pattern of amino acids in serum was normal except for a slight increase in the concentration of glutamic acid possibly due to contamination of glutamine during storage.

However a marked increase in the excretion of several amino acids such as hydroxy proline, proline, citrulline, alanine and lysine was found in both samples of urine (Table 2). Also the rate of excretion of tyrosine was abnormally high at the age of 21 months when the second urine specimen was obtained whereas a normal amount of this amino acid was found

Table 2 Amino acids in the urine

The values are given in mg per 24 hours

Amino acid	Specimens obtained at the age of		Normal range (ref 17)
	13 months	21 months	
Taurine	trace	7	96-254
Hydroxy proline	59	23	0-trace
Aspartic acid	13	2	10
Threonine	3	146	13-53
Serine	13	Not determined	27-73
Glutamic acid	555	45	8-40
Proline	367	319	10
Citrulline	40	44	—
Glycine	101	186	68-199
Alanine	307	193	21-71
Cysteine	29	38	10-21
Valine	trace	27	4-6
Methionine	trace	12	10
Isoleucine	8	trace	14-28
Leucine	4	7	9-26
Tyrosine	trace	118	13-49
Phenylalanine	trace	24	9-31
$\beta$ -amino acid	30	6	0-28
Ornithine	38	1	—
Lysine	534	310	7-48
Histidine	305	206	113-320
Arginine	trace	21	—

Table 1 Amino acid pattern in the serum

The values are given in  $\mu$ g per 100 ml

Amino acid	Specimens obtained at the age of		Normal range (ref 15)
	13 months	21 months	
Taurine	0.44	3.26	0.71-3.44
Aspartic acid	trace	0.78	0.05-0.27
Threonine	1.71	1.53	0.30-1.13
Serine	2.10	1.79	0.83-1.18
Glutamine	10.4	9.19	8.3-66.0
Asparagine	2.93	1.53	0.78-1.70
Proline	1.62	2.94	0.34-3.68
Citrulline	trace	0.57	0.21-0.52
Glycine	3.43	3.21	0.58-1.67
Alanine	4.90	3.80	1.2-2.72
Valine	2.48	2.05	1.30-3.31
Cysteine	trace	trace	0.34-0.91
Methionine	0.2	0.30	0.16-0.24
Isoleucine	0.73	0.77	0.37-1.1
Leucine	1.58	1.57	0.77-1.32
Tyrosine	0.80	0.89	0.59-1.28
Phenylalanine	2.30	1.76	0.43-1.02
Ornithine	1.27	1.73	0.36-1.14
Lysine	1.59	2.59	1.04-2.20
Histidine	0.61	1.27	0.37-1.12
Arginine	1.92	1.30	0.40-1.70

in the first specimen collected at the age of 13 months. On neither of these occasions could a generalized aminoaciduria be demonstrated since the amino acids not mentioned occurred in normal amounts (Table 2).

Table 3 shows calculated renal clearances of the various amino acids. Corresponding normal values for children at different ages reported by others (3, 11, 15) are presented for comparison. The data demonstrate the absence of a generalized aminoaciduria in our case. It has not been possible to derive exact renal clearances of hydroxy proline but they are very high for both specimens.

Interestingly at 13 months of age only the renal clearances of citrulline, glycine and cysteine were above those reported for healthy premature infants although when compared with clearance values valid for infants of more than 4 months of age the clearances of proline and lysine are also high. Correspondingly at the age of 21 months the renal clearances of hy-



Table 3 Renal clearances of amino acids

Values given as ml per min

Amino acid	Specimens obtained at the age of		Premature infants (Ref 11) <sup>a</sup>	Infants 16 days-4 months (Ref 3) <sup>b</sup>	Children	
	13 months	21 months			2-13 years (Ref 3) <sup>b</sup>	3-10 years (Ref 17)
Taurine	Not determined	0.4	0.6-1.3	—	—	9.9-26.2
Hydroxyproline	Not determined	High	16	—	—	Low
Aspartic acid	—	0.2	4.2 (1)	—	3.6±0.9	7.7-8.8
Threonine	0.03	7.6	3.0-8.2	2.0±1.4	1.0±0.2 <sup>c</sup>	0.5-2.5
Serine	Not determined	Not determined	4.6-9.3	4.1±1.9	2.4±0.5	1.2-3.4
Glutamic acid	2.9	1.1	—	—	—	0.1-2.4
Proline	1.8	9.7	2.1-13	1.0±0.7	0.4±0.2	0-0.3
Citrulline	High	5.3	0.6-2.5	—	—	0.6 (1)
Glycine	High	4.0	12-26	7.4±3.2	4.2±1.4	1.2-8.6
Alanine	0.9	3.5	1.5-3.7	1.7±0.8	0.8±0.4	0.2-1.3
Cystine	High	High	3.6-8.8 (2)	1.1±0.3	0.8±0.2	1.0-1.4
Valine	Low	1.0	0.2-0.6	0.3±0.1	0.2±0.1	0.1-0.3
Methionine	Low	0.3	2.3-5.8	0.8±0.6	0.8±0.3	1.0-3.4
Isoleucine	0.2	Low	0.4-1.0	0.3±0.2	0.3±0.1	0.2-1.0
Leucine	0.04	0.3	0.4-1.3	0.8±0.5	0.5±0.2	0.2-0.9
Tyrosine	Low	9.2	0.6-2.6	1.8±0.4	2.0±0.8	0.8-3.3
Phenylalanine	Low	0.9	1.2-2.2	1.7±0.9	1.5±0.3	0.3-2.3
Ornithine	0.4	0.04	0.9-1.0 (3)	0.4±0.1	0.4±0.1	0.2-0.8
Lysine	3.1	9.0	1.6-5.2	1.3±0.4	1.2±0.4	0.3-2.4
Histidine	7.0	11.2	7-19	8.5±4.5	9.5±2.6	1.9-21.8
Arginine	Low	1.1	—	0.4±0.1	0.3±0.1	0.2-1.2

<sup>a</sup> Values given represent range<sup>b</sup> Values given represent mean ± 3 SD<sup>c</sup> Threonine + Asparagine

Table 4 Amino acids in the cerebrospinal fluid

The values are given in  $\mu$ moles per 100 ml

Amino acids	Specimen obtained at age 13 months	Normals (Ref 4)
Taurine	0.52	0.22-0.94
Hydroxyproline	—	—
Aspartic acid	Not separated	0.03-0.22
Threonine	Not separated	0.77-4.91
Asparagine/ Glutamine	63.2	19.4-86.5
Serine	7.80	1.65-12.1
Glutamic acid	6.6	0.24-2.5
Proline	—	0-0.58
Citrulline	Trace	0.11-0.45
Glycine	1.0	0.40-1.07
Alanine	2.9	1.17-4.46
$\beta$ -aminobutyric acid	—	0.14-0.91
Cystine	Trace	Trace
Valine	2.2	0.74-2.82
Methionine	0.48	0.05-0.75
Isoleucine	0.65	0.30-0.86
Leucine	1.34	0.62-2.22
Tyrosine	1.20	0.30-2.43
Phenylalanine	1.40	0.16-2.74
Lysine	2.87	0.61-3.34
Arginine	2.62	0.80-2.81

droxy-proline, citrulline, cystine, valine, tyrosine and lysine are above those of premature infants but compared with normal infants older than 4 months elevated values are found for several other amino acids including threonine, serine, proline and alanine. Clearances of all other amino acids investigated were within normal limits for both samples.

Table 4 shows the amino acid concentrations in a sample of cerebrospinal fluid obtained at the age of 13 months. All amino acids studied appeared in normal concentrations, and the serum/CSF molar ratios were similar to those reported for normal adults (5).

## DISCUSSION

It has often been stated that the oculo-cerebro-renal syndrome is associated with generalized aminociduria (1). However, up to the present time few complete quantitative studies of urinary amino acids in this syndrome by reliable

methods such as ion exchange chromatography have been published. Furthermore even the published figures do not always confirm the presence of unselective renal losses of amino acids.

In our patient the aminoaciduria appears to be of a highly selective type with disproportionately high clearances for several amino acids including proline, lysine, hydroxy proline and citrulline. Furthermore the data suggest a progression of the aminoaciduria with time although an incidental variation in tubular dysfunction might account for the observed difference between the two samples. The data also confirm the renal origin of the aminoaciduria since serum amino acid concentrations were largely normal. The figures of Table 3 bear out a conspicuous similarity between the aminoaciduria observed in our patient and the 'physiologic' aminoaciduria of premature infants suggesting that tubular functional immaturity rather than specific toxic agents may be responsible for the aminoaciduria of patients with the Lowe syndrome.

In their review Abassi et al (1) reported quantitative data on amino acid concentrations in urine obtained from 7 patients with this syndrome and claimed the presence of generalized aminoaciduria. Their data show that the concentrations must be given in micromoles per 24 hours and not in millimoles per 24 hours as stated in the text since otherwise their normal controls would have had gross aminoaciduria. Furthermore in some of their cases the aminoaciduria appears to have been rather specific and selective and similar to that observed in our patient. Using paper chromatography Jagenburg (6) studied a type of fairly selective aminoaciduria in the Lowe syndrome similar to the aminoaciduria of classical cystinuria with prominent urinary losses of lysine, ornithine, arginine and cystine.

According to our present knowledge at least five group-specific enzyme systems are involved in the tubular reabsorptions of filtered amino acids (11). In our cases tubular transport of the aminoacids proline and hydroxy proline

as well as that of lysine and citrulline seems to be impaired whereas reabsorption of the neutral amino acids is largely normal. However in Jagenburg's case (6) reabsorption of the basic amino acids appeared to be specifically blocked. Thus the tubular lesion responsible for aminoaciduria in the Lowe syndrome may vary at least initially with respect to the particular enzyme systems involved. In our patient, the magnitude of pathologic  $\beta$ -microglobulinuria was similar to that previously reported in this syndrome as well as in other types of tubular dysfunction (13).

The bearing if any of the aminoaciduria on the co-existing tubular acidosis in the oculo-cerebro-renal syndrome remains unknown. In one case described by McCance et al (10) tubular ammonia production seemed to be deficient but confirmatory reports have not appeared. In our patient a low renal bicarbonate threshold appears to be responsible for the acidosis; this would correspond well to a proximal level of tubular dysfunction.

Since the Lowe syndrome involves the central nervous system and since the distribution of aminoacids between plasma and cerebrospinal fluid is believed to be dependent on active transport mechanisms concentrations of amino acids in a sample of cerebrospinal fluid were determined. As shown in Table 4 no abnormalities were found. On the other hand in some hydramencephalic patients studied by us distinctly abnormal cerebrospinal concentrations of various amino acids have been observed (18).

## SUMMARY

Ion exchange chromatographic studies of the distribution of a number of amino acids in the body fluids of a two-year-old boy with the oculo-cerebro-renal syndrome are reported. Amino acid concentrations in serum and in cerebrospinal fluid were normal whereas studies of the urine disclosed a highly selective (renal) aminoaciduria rather similar to that occurring in healthy premature infants.

Complementary observations on the acid-base metabolism served to establish the presence of renal tubular acidosis, probably due to a low tubular bicarbonate threshold.

The implications of these findings are briefly discussed.

## REFERENCES

- 1 Abusi V, Lowe C U & Ciccagno P L. Oculo-cerebro-renal syndrome. *Amer J Dis Child* 115 145 1968
- 2 Astrup P, Jørgensen K, Siggard Andersen O & Engel K. The acid-base metabolism. A new approach. *Lancet* i 1035 1960
- 3 Brodehl J & Gelman K. Endogenous renal transport of free amino acids in infancy and childhood. *Pediatrics* 42 395 1969
- 4 Dickinson J C & Hamilton P B. The free amino acids of human spinal fluid determined by ion exchange chromatography. *J Neurochem* 13 1179 1966
- 5 Hummoller F L, Mahler D J & Parker M M. Distribution of amino acids between plasma and spinal fluid. *Int J Neuropsych* 2 293 1966
- 6 Jørgensen O R. The urinary excretion of free amino acids and other amino compounds by the human. *Scand J Clin Lab Invest* 11 Suppl 43 150 1959
- 7 Jørgensen K. Titrimetric determination of the net excretion of acid base in urine. *Scand J Clin Lab Invest* 9 287 1957
- 8 Kildeberg P. *Clinical acid base physiology. Studies in neonates, infants and young children*. Munksgaard Copenhagen 1968
- 9 Lowe C U, Terrey M & McLachlan E. A. Organic aciduria. Decreased renal ammonia production, hydropothalamic and mental retardation. A clinical entity. *Amer J Dis Child* 94 164 1952
- 10 McCance K A, Matheson W J, Gresham G A & Ellington J R. The cerebro-ocular renal dystrophies. A new variant. *Arch Dis Child* 35 240 1960
- 11 O'Brien M & Butterfield L J. Further studies on renal tubular conservation of free amino acids in early infancy. *Arch Dis Child* 34 437 1963
- 12 Pallingaard G & Goldschmidt E. The oculo-cerebro-renal syndrome of Lowe in four generations of one family. *Acta Paediatr Scand* 1971
- 13 Peterson P A, Evrin P E & Berglund L. Differentiation of glomerular tubular and normal proteinuria. Determination of urinary excretion of  $\beta$ -microglobulin, albumin and total protein. *J Clin Invest* 48 1189 1969
- 14 Serner C R. Amino acid transport in the mammalian kidney. In W L Nyhan (ed) *Amino acid metabolism and genetic variation*. McGraw Hill New York 1967 p 327
- 15 Serner C R & Dives E. Endogenous renal clearance rates of free amino acids in prepubertal children. *Pediatrics* 36 592 1965
- 16 Sprickman D H, Stein W H & Moore S. Automatic recording apparatus for use in the chromatography of amino acids. *Anal Chem* 30 1191 1958
- 17 Stein W H. A chromatographic investigation of the amino acid constituents of normal W. *J Biol Chem* 201 45 1953
- 18 Ullén O, Hambræus L & Andersen G. The aminogram of cerebrospinal fluid in a case of hydranencephaly before and after shunt operation. In preparation

Submitted April 14 1970

Accepted April 28 1970

(L H) Perinatal Research Laboratory  
Akademiska Sjukhuset  
Uppsala  
Sweden

Key words: Lowe syndrome; amino acid metabolism

## FATTY ACID COMPOSITION OF PLASMA AND RED CELL PHOSPHOGLYCERIDES IN FULL TERM INFANTS AND THEIR MOTHERS<sup>1</sup>

RAONAR OLEGÅRD and LARS SVENNERHOLM

*From the Department of Paediatrics and the Department of Neurochemistry  
Psychiatric Research Center University of Göteborg Sweden*

There is evidence that malnutrition during early life can lead to persisting intellectual and emotional disturbances (2, 10). Almost all of the relevant studies have been concerned with protein-calorie deficiencies during the first period of life: nutritional status during the gestational period and the influence of other nutrients having received relatively little attention. Although the brain is built largely of lipids with a high concentration of polyunsaturated fatty acids which are essential for mammals, very little is known about the requirements of essential fatty acids for normal development of the foetal brain.

Knowledge about the supply of the human foetus with nutrients essential for normal development is incomplete. Between the maternal and foetal side of the placenta there are puzzling concentration gradients of many compounds: e.g. higher level of blood glucose and free amino acids at the foetal side (23). On the other hand it has long been known that the concentrations of plasma lipids are much lower in the newborn than in the mother (26).

No systematic studies of the serum fatty acid composition of well nourished mothers and their infants were known at the beginning of the present study of the blood phosphoglycer-

ides. This lipid class was chosen instead of total lipids in order to reduce the influence of rapid and transient changes in environmental conditions which were assumed to be reflected more instantly in the fatty acid composition of free fatty acids and triglycerides than in that of the phosphoglycerides.

### MATERIAL AND METHODS

#### *Clinical material*

The series consisted of 20 apparently healthy pregnant women. Ten of them were selected at random from the Prenatal Care Unit of the Obstetric Service, Sahlgrenska Hospital, Göteborg. The women were instructed to keep a dietary record for each week which was then checked at an interview by a trained dietitian. Their diets did not differ significantly from that of the general Swedish population. The remaining 10 women were selected at parturition. All of the women had uncomplicated pregnancies and deliveries. Seven of the women were primiparae, 9 were parae and 4 were triparae. The mothers' ages and the gestational ages are given in Table 1. The newborn infants showed normally respiratory and circulatory adaptation immediately after birth and uneventful neonatal courses. They were discharged from the hospital 4 to 6 days after birth. All the newborns were of full-term normal appearance except one with a gestational age of 42 weeks who looked somewhat dysmature. Birth-weights, lengths at birth and placental weights are given in Table 1.

#### *Chemicals*

*Solvents:* All organic solvents used were of reagent grade quality and freshly distilled before use. All ratios given in the text are on a volume basis.

*Adsorbents:* Silicic acid (Mallinckrodt A. R.) 300

<sup>1</sup> A preliminary report of the present data was given at the Senior Symposium of Nutrition, Wenner Gren Centre, Stockholm, 1968.

Complementary observations on the acid base metabolism served to establish the presence of renal tubular acidosis, probably due to a low tubular bicarbonate threshold

The implications of these findings are briefly discussed

# REFERENCES

- 1 Abassi V, Lowe C U & Calcagno P L. Oculo cerebro renal syndrome *Amer J Dis Child* 115 145 1968
- 2 Astrup P, Jørgensen K, Sjøgaard Andersen O & Engel K. The acid-base metabolism. A new approach *Lancet* 1 1035 1960
- 3 Brodehl J & Gelersen K. Endogenous renal transport of free amino acids in infancy and childhood *Pediatrics* 42 395 1968
- 4 Dickinson J C & Hamilton P B. The free amino acids of human spinal fluid determined by ion exchange chromatography *J Neurochem* 13 1179 1966
- 5 Hummoller F L, Mahler D J & Parker M M. Distribution of amino acids between plasma and spinal fluid *Int J Neuropsych* 2 293 1966
- 6 Jørgensen O R. The urinary excretion of free amino acids and other amino compounds by the human *Scand J Clin Lab Invest* 11 Suppl 43 150 1959
- 7 Jørgensen K. Titrimetric determination of the net excretion of acid base in urine *Scand J Clin Lab Invest* 9 287 1957
- 8 Kuldeberg P. *Clinical acid base physiology. Studies in neonates, infants and young children*. Munksgaard Copenhagen 1968
- 9 Lowe C U, Terrey M & McLachlan E. A. Organic aciduria. Decreased renal ammonia production, hydrocephalus and mental retardation. A clinical entity *Amer J Dis Child* 84 164 1952
- 10 McCance R A, Matheson W J, Gresham G

- A & Elkinton J R. The cerebro-ocular renal dystrophies. A new variant *Arch Dis Child* 35 240 1960
- 11 O'Brien D & Butterfield L J. Further studies on renal tubular conservation of free amino acids in early infancy *Arch Dis Child* 38 437 1963
- 12 Pallingaard G & Goldschmidt E. The oculo-cerebro renal syndrome of Lowe in four generations of one family *Acta Paediatr Scand* 1971
- 13 Peterson P A, Eyrin F E & Bergsjeld I. Differentiation of glomerular tubular and normal proteinuria. Determination of urinary excretion of  $\beta$ -microglobulin, albumin and total protein *J Clin Invest* 48 1189 1969
- 14 Scriver C R. Amino acid transport in the mammalian kidney. In W L Nyhan (ed) *Amino acid metabolism and genetic variation*. McGraw Hill New York 1967 p 327
- 15 Scriver C R & Davies E. Endogenous renal clearance rates of free amino acids in prepubertal children *Pediatrics* 36 392 1965
- 16 Spackman D H, Stein W H & Moore S. Automatic recording apparatus for use in the chromatography of amino acids *Anal Chem* 31 1191 1958
- 17 Stein W H. A chromatographic investigation of the amino acid constituents of normal urine *J Biol Chem* 101 45 1953
- 18 Ulfvén O, Hambræus L & Andersson G. The aminogram of cerebrospinal fluid in a case of hydranencephaly before and after shunt operation. In preparation

Submitted April 14 1970  
Accepted April 28 1970

(L H) Perinatal Research Laboratory  
Akademiska Sjukhuset  
Uppsala  
Sweden

Key words: Lowe syndrome, amino acid metabolism

## FATTY ACID COMPOSITION OF PLASMA AND RED CELL PHOSPHOGLYCERIDES IN FULL TERM INFANTS AND THEIR MOTHERS<sup>1</sup>

RAGNAR OLEGARD and LARS SVENNERHOLM

*From the Department of Pediatrics and the Department of Neurochemistry  
Psychiatric Research Center University of Göteborg Sweden*

There is evidence that malnutrition during early life can lead to persisting intellectual and emotional disturbances (2-10). Almost all of the relevant studies have been concerned with protein-calorie deficiencies during the first period of life. Nutritional status during the gestational period and the influence of other nutrients have not received relatively little attention. Although the brain is built largely of lipids with a high concentration of polyunsaturated fatty acids which are essential for mammals, very little is known about the requirements of essential fatty acids for normal development of the fetal brain.

Knowledge about the supply of the human foetus with nutrients essential for normal development is incomplete. Between the maternal and foetal side of the placenta there are puzzling concentration gradients of many compounds: a higher level of blood glucose and free amino acids at the foetal side (23). On the other hand it has long been known that the concentrations of plasma lipids are much lower in the newborn than in the mother (26).

No systematic studies of the serum fatty acid composition of well nourished mothers and their infants were known at the beginning of the present study of the blood phosphoglycer-

ides. This lipid class was chosen instead of total lipids in order to reduce the influence of rapid and transient changes in environmental conditions which were assumed to be reflected more instantly in the fatty acid composition of free fatty acids and triglycerides than in that of the phosphoglycerides.

### MATERIAL AND METHODS

#### *Clinical material*

The series consisted of 20 apparently healthy pregnant women. Ten of them were selected at random from the Prenatal Care Unit of the Obstetric Service 1 Sahlgrenska Hospital, Göteborg. The women were instructed to keep a dietary record for each week which was then checked at an interview by a trained dietitian. Their diets did not differ significantly from that of the general Swedish population. The remaining 10 women were selected at parturition. All of the women had uncomplicated pregnancies and deliveries. Seven of the women were primiparae, 9 were multiparae and 4 were triplet. The mothers' ages and the gestational ages are given in Table 1. The newborn infants showed normally respiratory and circulatory adaptation immediately after birth and uneventful neonatal courses. They were discharged from the hospital 4 to 6 days after birth. All the newborns were of full-term normal appearance except one with a gestational age of 42 weeks who looked somewhat dysmature. Birthweights, lengths at birth and placental weights are given in Table 1.

#### *Chemicals*

**Solvents.** All organic solvents used were of reagent grade quality and freshly distilled before use. All ratios given in the text are on a volume basis.

**Absorbents.** Silicic acid (Mallinckrodt A. R.) 200

<sup>1</sup> A preliminary report of the present data was given at the Sempser Symposium of Nutrition, Wenner Gren Centre, Stockholm, 1968.

Table 1 Age and period of pregnancy of the mothers, birth weight and length of the newborns and placental weights

Number—20	Mean	Range
<b>Mothers</b>		
Age	27 years	17–36 years
Gestational age	40.4 weeks	40–42 weeks
<b>Newborns</b>		
Birthweight	3 314 g	2 790–3 990 g
Length at birth	49.3 cm	47–53 cm
Placental weight	537 g	400–780 g

meat was pressed through a 325 sieve, freed from impurities and fines by suspension in methanol and activated at 120°C (52). A 0.25 mm layer of Silica Gel (E. Merck A. G. Darmstadt) was spread on a glass plate 20×20 cm and activated overnight at 120°C.

#### Quantitative determination of lipids

Lipid phosphorus was assayed by a Bartlett procedure (3), cholesterol by the method of Crawford (11) and triglycerides by the method of Carlson (8). Phospholipid concentration was calculated by multiplying the value for lipid phosphorus by 25 (51).

#### Blood sampling

Immediately after the cord had been clamped, blood from the placental stump of the umbilical cord was allowed to run freely into a heparinized test tube, care being taken to avoid admixture of maternal blood. The sample was collected while the placenta was still in the uterus. A maternal venous blood sample was obtained from an antecubital vein within 5 min of delivery.

The plasma was separated from the blood cells within 4 hours by centrifugation. The plasma was removed by suction and the red cells were washed twice with 2 to 3 volumes of physiological saline. After the last centrifugation at 2 500×g for 10 min, 0.5 ml of the packed and washed red cell sediment was transferred to a new test tube. The red cells were then hemolyzed by addition of 10 ml of 0.005 M phosphate buffer, pH 6.8, and the tube centrifuged at 3 000×g for 60 min. The red and clear supernatant was removed from the slightly pink sediment of the red cell stroma.

#### Preparation of lipid extracts

Six ml of C.M. 1:1 was added to the test tube with red cell stroma, which was then shaken vigorously. In the same way 6 ml of C.M. 1:1 was added to 0.5 ml of plasma. The tube was centrifuged at 2 000×g for 10 min and the extract was transferred to a tube with ground glass stopper. The sediment was removed with 2 ml of C.M. 1:1. The two extracts were pooled and 4 ml of chloroform and

2 ml of acid physiological saline (0.5 g sodium chloride and 0.5 ml 1 M sulfuric acid dissolved into 500 ml of distilled water) was added after which the tube was vigorously shaken for 1 min and centrifuged (2 000×g) for 10 min. The upper phase was removed and the lower phase was washed with running solution (15 ml of chloroform, 260 ml of methanol and 250 ml of 0.1% sodium chloride) and then transferred to a 10 ml cylinder. The volume was made up to exactly 10 ml with C.M. 2:1. Duplicate aliquots of 0.2 ml were used for determination of the lipid phosphorus.

#### Separation of lipids by column chromatography with silicic acid

The lipid extract (9.6 ml) was transferred to a round bottomed flask and evaporated to dryness under reduced pressure in a rotating evaporator at a temperature not exceeding 40°C. The lipids were immediately redissolved in 5 ml of chloroform. The chloroform extract was added to a column containing 1 g of silicic acid. The column was prepared by suspending the silicic acid into chloroform and stirring it into a glass column with an inner diameter of 6 mm. The flask was rinsed twice with 2 ml of chloroform which was added to the column. When the lipid extract had entered the column, the elution of neutral lipids was completed by adding 15 ml of C.M. 19:1 for plasma and 15 ml of C.M. 50:1 for red cell stroma. The effluent was collected into a 25 ml cylinder. The plasma phospholipids were eluted with 25 ml of methanol. The red cell phospholipids were separated into cephalins and choline phospholipids. The former were eluted by 25 ml of C.M. 3:1 and the latter by 25 ml of methanol. The separation was checked by thin layer chromatography on a silica gel plate which was developed with C.M.W. 65:25:4. The spots were visualized by spraying with anisaldehyde solution (36). Duplicate aliquots of the neutral lipid eluates were used for quantitative determination of triglycerides and cholesterol and duplicate aliquots of the phospholipid eluates for quantitative determination of phospholipids. Aliquots of the phospholipid eluate equivalent to about 10 µg of lipid phosphorus were evaporated to dryness under nitrogen in conical tubes with ground glass stoppers.

#### Separation of lipids by thin layer chromatography on silica gel

Portions of the plasma lipid extract corresponding to 10–20 µg of lipid phosphorus were applied as 2–3 cm broad bands on 20×20 cm thin layer plates coated with silica gel. The plates were developed with C.M.W. 65:25:4 for the isolation of lecithins and with light petroleum-diethyl ether-acetic acid 90:10:1 for isolation of total plasma phosphoglycerides. The spots were visualized by spraying with bromothymol blue reagent (31). The spots with lecithins or total phosphoglycerides respectively were quantitatively scraped into glass tubes and placed into a vacuum desiccator which was immediately evacuated and allowed to stand over night (53).

*analysis of phosphoglycerides*

preparation of methyl esters of the phosphoglycerides and their quantitative assay by GLC were carried with the standard technique of the laboratory. Two ml of 0.1 M sodium methoxide in dry methanol was added and the tubes were gently shaken 1 hour at room temperature. For neutralization 0.1 ml of 1 M acetic acid was added and the methyl esters were extracted three times with 2 ml of light petroleum b.p. 45–50°C. The light petroleum extracts were pooled in conical tubes with ground glass joints and washed with distilled water. The water removed from the bottom by syringe. To remove water the tubes were frozen to –20°C and the petroleum decanted off into new tubes.

*GC of methyl esters*

The extract with the fatty acid methyl esters was poured to about 100 µl on a water bath at 25°C for a gentle stream of nitrogen and then transferred to a chemical microtube (volume 500 µl) with glass pipette. It was then evaporated further to a final size of about 10 µl. One µl was analysed in a Finn Elmer Model 880 apparatus on a 200 cm stainless steel column of 3/32 in. Ø packed with dactylphenyl glycol succinate polyester (DEGS) and on Gaschrom Q 80–100 mesh Helmax was used as carrier gas and the column temperature was 200°C. The peaks in the chromatograms were identified and quantitated in the way described previously (7).

*Statistical methods*

The mean standard deviation of the concentrations of plasma lipid classes and of the fatty acid concentrations in phosphoglycerides were calculated since it cannot be postulated that the values for mothers and newborns are independent; the statistical significance of the differences was assessed from the *F*-values of fatty acid concentrations in the particular parts of mother-newborns. It was postulated that the differences in concentrations were normally distributed. For the correlation analyses it was supposed that the distribution of fatty acid concentrations and concentrations of cholesterol and phospholipids in mothers and newborns constituted two-dimensional normal distributions (48).

## RESULTS AND DISCUSSION

*Concentrations of main lipid classes in plasma*  
The mean values and standard deviations found for the plasma lipids are given in Table 2. The total lipids were calculated as the sum of the different lipid classes. The cholesterol figure was multiplied by 1.44 to include the fatty acids in the cholesterol esters and for free fatty acids figures of 20 mg/100 ml and 10 mg/

Table 2 Mean concentration of plasma lipids at birth

Constituent	Mother (mg/100 ml) (n = 20)		Infant (mg/100 ml) (n = 20)	
	Mean	S.D.	Mean	S.D.
Total lipids	886	126	257	99
Triglycerides	179	58	39	20
Cholesterol	257	39	66	18
Phospholipids	317	36	113	24
Cholesterol/ phospholipid ratio	0.81	0.06	0.99	0.07

100 ml were added for mothers and newborns respectively.

*Mothers* The mean cholesterol concentration of 257 mg/100 ml is in good agreement with the mean of earlier investigations of 272 mg/100 ml (4, 7, 14, 21, 22, 29, 30, 32–35, 44, 47, 54, 57). The range of the means in earlier investigations was wide 208–357 mg/100 ml but in 11 of the 17 studies it was 240 to 275 mg/100 ml. The total phospholipid concentration of 317 mg/100 ml found in the present study also agrees well with the mean of earlier investigations of 306 mg/100 ml range 192–406 mg/100 ml (13, 14, 21, 22, 30, 32, 34, 42, 44, 46, 58). The phospholipid concentration of 406 mg/100 ml reported by Renkonen (44) from Finland is the highest on record and might be due to some unknown sources of error because at this concentration the cholesterol/phospholipid quotient would be very low 0.64. In previous studies a mean cholesterol/phospholipid quotient of 0.89 can be calculated which is slightly higher than the present ratio of 0.81. The present value for triglycerides of 179 mg/100 ml is lower than the mean found in earlier studies viz. 249 mg/100 ml range 159–302 mg/100 ml (13, 14, 32, 34, 44, 47, 54). No relation was found in earlier publications between the time of collection of the blood (just before or after delivery) and the level of triglycerides.

*Newborns* The present values for triglycerides, cholesterol and total phospholipids are in good agreement with the mean values ob-



Table 3 *Fatty acid composition of infant blood plasma phosphoglycerides isolated by three different methods*

Fatty acid	Phosphoglycerides (n=10)				Lecithin (n=10)		Differences (B)-(A)		Differences (C)-(A)	
	Column (A)		TLC (B)		TLC (C)					
	Mean	S D	Mean	S D	Mean	S D	Mean		Mean	
16:0	29.6*	0.49	30.9	0.98	30.9	1.74	+1.2	**	+1.2	**
16:1	1.7	0.28	1.6	0.33	1.6	0.44	0.0	-	-0.2	-
18:0	14.7	0.96	15.0	1.20	14.3	0.80	+0.3	-	-0.5	**
18:1	12.7	1.33	12.8	1.34	12.7	1.53	0.1	-	0.0	-
18:2 (n=6)	8.5	1.52	8.7	1.36	8.8	1.16	+0.2	-	+0.3	-
20:3 (n=6)	5.5	0.72	5.4	0.86	5.7	0.71	-0.1	-	+0.1	-
20:4 (n=6)	16.6	1.72	15.4	1.59	15.7	1.82	-1.1	**	-0.7	*
22:6 (n=3)	7.2	1.19	6.8	1.03	7.2	1.88	-0.4	-	+0.1	-
18-22 (n=6)	32.2	1.21	30.9	1.80	31.5	1.63	-1.2	**	-0.7	-

\* Values are weight percentages of methyl esters

\* Stands for significance level -0.05 level \* -0.01 level \*\* -0.001 level

tuned in earlier investigations for triglycerides 50 mg/100 ml range 33-87 mg/100 ml (5, 6, 32, 34, 44, 54) for cholesterol 73 mg/100 ml, range 34-98 mg/100 ml (4-7, 12, 19-22, 25, 29, 30, 32-35, 38, 40, 43-45, 49, 54, 59) and for total phospholipids 119 mg/100 ml, range 61-212 mg/100 ml (5, 6, 9, 12, 19-22, 30, 32, 34, 38, 40, 42-45, 54, 59). The cholesterol/phospholipid ratio in the aforementioned earlier studies as calculated by us was 0.66 compared with 0.59 in the present study. The present figures for cord blood were very close to those in previous Scandinavian studies by

Brody & Carlsson (6) and by Renkonen (44). No correlation was found between the plasma cholesterol or phospholipid levels in the mothers and their newborns. The triglycerides were not studied for any such correlation it being known that they have a skew distribution (6).

#### *Fatty acid composition of blood phosphoglycerides*

*Comparison of methods.* A. Isolation of plasma phosphoglycerides: There are two principal methods for the isolation of total plasma phospholipids: column and thin layer chromatography.

Table 4 *Fatty acid composition of mother blood plasma phosphoglycerides isolated by three different methods*

Fatty acid	Phosphoglycerides (n=10)				Lecithin (n=10)		Differences (C)-(A)	
	Column (A)		TLC (B)		TLC (C)			
	Mean	S D	Mean	S D	Mean	S D	Mean	
16:0	30.1	1.48	31.1	1.01	32.1	1.57	+2.1	**
16:1	1.6	0.30	1.5	0.37	1.5	0.42	-0.1	-
18:0	11.4	1.06	11.4	0.97	10.0	0.98	-1.5	*
18:1	14.2	1.47	14.8	1.53	14.4	1.50	0.2	-
18:2 (n=6)	22.2	2.82	22.2	3.20	23.4	3.15	+1.2	*
20:3 (n=6)	3.2	0.54	3.1	0.45	3.2	0.56	0.0	-
20:4 (n=6)	8.9	1.42	8.2	1.29	7.8	1.51	-1.0	*
22:6 (n=3)	5.6	0.82	5.1	0.63	4.9	0.72	-0.7	*
18-22 (n=6)	34.9	2.57	34.0	3.15	35.0	2.56	+0.2	-

\* and \* see text in Table 3

graphy. The latter method has been more widely used during the last few years because it is simpler and more sensitive. When mild alkaline transmethylation is used only the phosphoglyceride fatty acids will be transmethyated. Since different phosphoglycerides have their own characteristic fatty acid pattern there is also an increasing tendency to determine the fatty acid composition of individual phosphoglycerides. Lecithin is the predominating phosphoglyceride of plasma and constitutes more than 90% of the total plasma phosphoglyceride fatty acids (41). We therefore thought it worth while to find out whether the two isolation procedures for total plasma phosphoglycerides and for lecithin gave comparable fatty acid data. Phospholipids from 10 individual samples of maternal plasma and umbilical cord plasma were isolated by three different methods: on silica acid column according to the procedure described (A), by thin layer chromatography in *h<sub>18</sub>* petroleum-ethyl ether-acetic acid 90:10:1 v/v/v and isolation of the total phospholipid fraction (B) and by thin layer chromatography in chloroform-methanol-water 65:25:4 v/v/v and isolation of the lecithin spot (53). The results are given in Tables 3 and 4. When the total phospholipids were isolated by column and thin layer chromatography some significant differences were found for the fatty acid composition of the infant blood plasma phosphoglyceride fraction. The TLC procedures gave lower concentrations of 20:4 ( $n=6$ ) and 22:6 ( $n=3$ ). In the TLC method (B) the phospholipids remained at the starting point together with non-lipid contaminants. It is probable that heavy metals and particularly hemoglobin derivatives caused an auto-oxidation of the two above mentioned highly polyunsaturated fatty acids (24). In a previous study (53) no difference was found in the brain phosphoglyceride fatty acid pattern whether antioxidants were added to the lipid extracts or not, but it is possible that such an addition would have had some effect in this study because the phospholipids did not migrate from the contaminants. The differences in fatty acid distribution between total

phosphoglycerides and lecithins in umbilical cord blood were so small (Table 3) that the methods can be used interchangeably.

In the isolation of total phospholipids in maternal plasma thin layer chromatography (B) gave lower values for 20:4 ( $n=6$ ) and 22:6 ( $n=6$ ) than did column chromatography method (A), just as it did for umbilical cord plasma. Since the percentages of these acids are lower in the maternal plasma the difference may be ignored. The fatty acid pattern of total phosphoglycerides (A) and that of lecithin (C) differed slightly more in the maternal plasma than in the infant's plasma. In lecithin 16:0 and 18:2 were higher and 20:4 ( $n=6$ ) was lower than in the total phosphoglycerides ( $p < 0.001$ ). However, the standard deviation of the individual fatty acid levels was much lower than in previous studies (44-58) so that the three methods can be used interchangeably also for the maternal plasma.

**B. Extraction of red cell lipids.** The lipids of red cells were extracted from saline washed intact cells by the procedure III method of Ways & Hanahan (55) or from red cell stroma obtained by lysis in weak phosphate solution (see under Methods). Extraction of red cell stroma instead of intact red cells was faster and easier and the volume of extraction solvent could be strongly reduced. The yield of total phospholipids was the same or slightly better from the stroma than from the intact cells. The fatty acid patterns were very similar with the two methods but in most of the samples the concentration of polyenoic acids were somewhat higher in the stroma extracts. This difference was most pronounced for the cephalin fraction.

It was thus evident that the extraction of red cell stroma instead of intact cells had several advantages and reduced the risk for oxidation catalysed by heme derivatives of the most polyunsaturated fatty acids.

**Maternal plasma phosphoglycerides and red cell lecithins.** The mean and standard deviations of the fatty acid composition of plasma phosphoglycerides are given in Table 5. The highest concentrations were found for 16:0

Table 5 Fatty acid composition of plasma phosphoglycerides and red blood cell lecithin in mother and infant

Fatty acid	Plasma phosphoglycerides			Red blood cell lecithin		
	Mother (n=20)	Infant (n=20)	Difference Infant Mother	Mother (n=10)	Infant (n=9)	Difference Infant Mother
	Mean S D	Mean S D	Mean	Mean S D	Mean S D	Mean
16 0	31.3 <sup>a</sup> 2.26	30.2 1.37	-1.1 <sup>ab</sup>	36.4 1.86	36.5 2.02	+0.2 <sup>a</sup>
18 1	1.4 0.40	1.5 0.38	+0.1 <sup>-</sup>	1.6 0.51	1.6 0.58	-0.1 <sup>-</sup>
18 0	11.5 0.92	15.2 1.05	+3.7 <sup>*</sup>	10.2 0.93	11.9 0.79	+1.8 <sup>ab</sup>
18 1	14.3 1.52	12.1 1.31	-2.1 <sup>***</sup>	19.4 1.29	18.8 1.70	-0.6 <sup>-</sup>
18 2 (n=6)	22.1 2.54	8.8 1.31	-13.3 <sup>***</sup>	18.1 2.40	7.8 1.50	-10.2 <sup>***</sup>
18 3 (n=3)+20 1	1.0 0.28	0.4 0.11	-0.6 <sup>*</sup>	1.2 0.30	0.4 0.14	-0.8 <sup>ab</sup>
20 3 (n=9)	0.2 0.09	0.6 0.39	+0.4 <sup>**</sup>	Traces	0.3	
20 3 (n=6)	3.1 0.54	5.5 0.75	+2.3 <sup>***</sup>	2.3 0.50	3.9 0.63	+1.6 <sup>*</sup>
20 4 (n=6)	8.5 1.34	16.7 1.40	+8.2 <sup>***</sup>	6.4 0.55	12.4 1.31	+6.0 <sup>*</sup>
20 5 (n=3)	0.7 0.19	0.4 0.31	-0.3 <sup>*</sup>	0.5 0.25	0.3 0.19	-0.2 <sup>-</sup>
22 4 (n=6)	0.2 0.09	0.7 0.15	+0.4 <sup>**</sup>	0.4 0.08	0.8 0.26	+0.4 <sup>*</sup>
22 5 (n=6)	0.3 0.14	0.6 0.24	+0.2 <sup>*</sup>	0.3 0.32	0.5 0.28	+0.2 <sup>-</sup>
22 5 (n=3)	0.6 0.17	0.5 0.19	0.0 <sup>-</sup>	0.5 0.20	0.4 0.14	-0.1 <sup>-</sup>
22 6 (n=3)	4.8 1.06	6.8 1.39	+2.0 <sup>*</sup>	2.5 0.73	4.2 1.12	+1.8 <sup>*</sup>
18-22 (n=6)	34.3 2.48	32.4 1.65	-2.0 <sup>-</sup>	27.6 2.06	25.4 1.77	-2.0 <sup>-</sup>
18-22 (n=3)	7.1 1.15	8.1 1.46	+1.0 <sup>**</sup>	4.6 0.90	5.4 1.33	+0.7 <sup>-</sup>

<sup>a</sup> and <sup>b</sup> see text in Table 3

18 2, 18 1 and 18 0 in decreasing order. The fatty acid composition of phosphoglycerides of maternal plasma at term has so far been determined only by Renkonen (44) and Zee (58), who studied plasma lecithin. Direct comparison of fatty acid data can often be only approximate because the percentage composition of the fatty acids depends on the number of fatty acids recorded. The former two investigators analysed a smaller number of fatty acids than we. The values found by them are in general agreement with ours, but they found a lower concentration of linoleic acid and the other polyunsaturated fatty acids, and a higher concentration of saturated and monoenic acids than we. Since the differences were largest for the most polyunsaturated fatty acids, a certain loss of these fatty acids during isolation in their studies is a more probable explanation than any difference in the dietary supply of polyunsaturated fatty acids.

The figures recorded for maternal plasma phosphoglycerides agreed closely with those found in studies in normal adults, mainly males (24, 39, 46, 59) though Phillips & Dodge (41)

found slightly higher figures for 18 2 and 20 4. In a study of adult males (1) in our laboratory with exactly the same method and performed at the same time as the present investigation 18 2 was higher and 20 4 was lower than in the maternal plasma. It is not possible to assess the significance of the small differences found in these two studies because the physiological variation of the phosphoglyceride fatty acid patterns with age, sex, season, diet etc. are not known.

The maternal red cell lecithins contained roughly the same percentages of 20 4 and 22 6 as did those found in earlier studies of red blood cells in normal adults (16, 55, 56). The concentration of 20 3 (n=6) was slightly higher in the maternal samples while the concentration of 18 2 was somewhat lower. Red blood cell lecithin contained considerably lower concentrations of 18 2, 20 4 and 22 6 than did plasma phosphoglycerides and higher concentrations of 18 1 and 16 0. This is in accordance with the results found by Dodge & Phillips (16) for five adult males.

In the present study a significant correlation

Table 6 Fatty acid composition of red blood cell cephalins in mother and infant

Fatty acid	Mother (n=10)		Infant (n=9)		Difference Infant-Mother	
	Mean	S.D.	Mean	S.D.	Mean	
16:0	13.9 <sup>a</sup>	1.14	15.4	2.58	+1.7	*
16:1	1.0	0.22	1.2	0.36	+0.2	
18:0	19.7	1.20	20.6	0.93	+0.9	
18:1	17.0	0.78	12.3	1.01	-5.0	
18:2 (n-6)	4.2	0.43	1.8	0.36	-2.4	*
18:3 (n-3)+20:1	1.2	0.17	0.5	0.14	-0.7	
20:3 (n-5)	Traces		0.8	0.20		
20:3 (n-6)	1.6	0.22	2.3	0.26	+0.7	
20:4 (n-6)	20.8	0.99	24.3	1.89	+3.5	
20:5 (n-3)	1.0	0.28	0.3	0.12	-0.8	-
22:4 (n-6)	4.1	0.40	6.6	1.00	+2.6	
22:5 (n-6)	0.8	0.31	1.8	0.52	+1.1	
22:5 (n-3)	3.8	0.52	1.4	0.33	-2.6	
22:6 (n-3)	10.7	0.86	10.8	1.10	+0.1	-
22:22 (n-6)	31.6	1.70	34.9	2.34	+3.3	
18-22 (n-3)	16.8	1.49	12.9	1.36	-3.9	

<sup>a</sup> and <sup>b</sup> see text in Table 3

( $r=0.768$ ) ( $n=10$ ) at the 0.01 level was found between the concentration of 18:2 in maternal plasma phosphoglycerides and that in red cell lecithin. There was no significant correlation at the 0.05 level ( $r=0.357$ ) between the concentration of 20:4 in maternal plasma phosphoglycerides and that in red cell lecithin.

**Newborns' plasma phosphoglycerides and red cell lecithin.** The fatty acid composition of umbilical cord plasma phosphoglycerides (Table 5) corresponds well with that found by Zöllner (39) in 7 cord sets although 22:6 ( $n=3$ ) was lower in their study. Renkonen (44) and Zee (58) found lower figures for 20:4 and 22:6 (the latter acid was not determined by Zee).

The fatty acid composition of umbilical cord red cell lecithin differed from that of plasma in exactly the same way as that of the maternal plasma and red blood cell. 18:2, 20:4 and 22:6 were lower and 16:0 and 18:1 were higher in red cell lecithin than in plasma phosphoglycerides. The fatty acid pattern found in the present study agreed well with that reported by Crowley, Ways & Jones (12).

There was a significant correlation between concentration of linoleic acid in plasma phosphoglycerides and that in red cell lecithin at

the 0.01 level ( $r=0.803$ ) ( $n=10$ ) and for arachidonic acid at the 0.05 level ( $r=0.722$ ) ( $n=10$ ).

**Mothers and newborns red cell cephalins.** The cephalins differed from lecithin by a high level of arachidonic acid and a very low level of linoleic acid. The ratio between the two fatty acids was 5:1 for cephalin compared to 1:3 for lecithin of the maternal red cells and 12:1 and 1.5:1 respectively for the newborns cells. The sum of the fatty acids of the linoleic acid series 18-22 ( $n=6$ ) was slightly higher in the maternal red cell cephalins than lecithin but this difference was still higher in the same lipid fractions of the newborns. The sum of the fatty acids of the linoleic acid series was significantly higher at the 0.001 level in the infants' red cell cephalins than in the mothers.

The level of the most polyunsaturated fatty acids of the linoleic acid series was manifold larger in the red cell cephalins than in lecithin of the mothers while the difference was somewhat less in the newborns. The concentration of 22:6 ( $n=3$ ) was the same in the mothers and infants' red cell cephalins but 22:5 ( $n=3$ ) was significantly larger in mothers' red cell cephalins. The concentration of all polyunsaturated fatty acids was slightly higher in the

newborns than in the mothers' red cell cephalins, 50% and 48% respectively.

There are few reports on the fatty acid composition of red cell cephalins in the literature (16, 17, 55, 56). The present figures for the fatty acid composition of mothers' red cell cephalins show a very good agreement with the figures of Dodge & Phillips (16) for ethanolamine and serine phosphoglycerides of male adult red cells. In our study 16:1 was larger, 20:4 ( $n=6$ ) smaller and 22:6 ( $n=3$ ) larger than in their study, but the differences were small. The fatty acid composition of the newborns' red cell cephalins in this study is very similar to that found by Crowley et al. (12) for newborns' ethanolamine plus serine phosphoglycerides.

**Mother-infant relation.** The sum of all fatty acids of the linoleic acid series in plasma phosphoglycerides and red cell lecithin in the infants' blood was only slightly lower than in the mothers' blood, and the differences were significant only at the 0.01 level. In the cephalins this sum was about 25% higher in the newborns than in the mothers, the difference significant at the 0.001 level. The concentration of linoleic acid 18:2 ( $n=6$ ) was much lower in both plasma and red blood cells of newborns' blood than of maternal blood (Tables 5-6). The lower 18:2 concentration of the cord blood was associated with much higher concentrations of the more unsaturated fatty acids of the linoleic acid series, mainly 20:4 ( $n=6$ ) and 20:3 ( $n=6$ ). These findings are in complete agreement with previous reports (44, 50, 58). A correlation was found between the concentrations of linoleic acid in maternal and cord plasma phosphoglycerides, significant at the 0.05 level ( $r=0.478$ ,  $n=20$ ).

The concentration of fatty acids of the linolenic acid 18:3 ( $n=3$ ) series was higher in cord blood plasma phosphoglycerides and red cell lecithin than in the mothers' blood. By contrast, in the cephalins the sum of the fatty acids of the linolenic acid series was significantly higher in the mothers' red cells than in the infants' (Table 6). The pattern of the fatty acids of this

series showed the same tendency as the linoleic acid series. The level of the parent fatty acid 18:3 ( $n=3$ ) was lower in the infants than in the mothers' plasma phosphoglycerides and red cell lecithin, but the more polyunsaturated acids were increased. The figures for 18:3 ( $n=3$ ) are however uncertain because 18:3 ( $n=3$ ) was not separated from 20:1 with the polar column used for GLC separation of fatty acids.

The present study demonstrated very small individual variations in the blood phosphoglyceride fatty acid pattern of mothers in labour and their infants. The small variations support the view that environmental factors play at most a minor role compared to a homeostatic mechanism for the regulation of the fatty acid pattern of blood phosphoglycerides. The influence of dietary factors on plasma lipids and fatty acids of mothers and their infants was studied by Hunsen et al. (25). They found no correlation between mother's intake of linoleic acid, varying from 1.5 to 8.6% of calories, and the serum dienoic and tetraenoic acid levels of her infant at birth. Renkonen (44) found substantially lower concentrations of the fatty acids of the linoleic acid series in plasma phosphoglycerides of Finnish mothers and their infants. He ascribed the low levels of fatty acids of the linoleic acid series to the large proportion of milk fats poor in linoleic acid in the Finnish diet. A low recovery of all the polyunsaturated fatty acids better explains his results.

In essential fatty acid deficiency 20:3 ( $n=9$ ) is increased (18, 28, 37). The concentration of this acid was very low in our normal adults, but the level was significantly higher in the newborns' blood, and a slight relative lack of essential fatty acids of the newborns cannot be excluded. The most polyunsaturated fatty acids of the linoleic and linolenic acid series were however much higher in the infants than in the mothers' blood, and the increase of 20:3 ( $n=9$ ) of the oleic acid series might only reflect a generalized increased desaturation and chain elongation of the fatty acids also in this series.

The inverted 18:2/20:4 ratio of the lecithin and cephalins in newborns' blood com-

pared with that in maternal blood might be explained by assumption of a facilitated transport of 20:4 prior to that of 18:2 from the mother to the foetus or by an increased conversion rate of 18:2 to 20:4 in the foeto-placental organism. A selective transport of linoleic acid across the placenta has been reported in the guinea pig (27) in which animal in contrast to that seen in humans the percentage of linoleic acid in plasma free fatty acids was approximately 50% greater in the foetus than in the mother. On the other hand, an increased activity in the foeto-placental organism of the desaturating and chain elongating enzyme systems which convert the parent fatty acids linoleic and linolenic acids to the more unsaturated  $C_{18}$  and  $C_{20}$ -acids might also explain the changed fatty acid pattern.

The physiological significance of the increased unsaturation of the fatty acids in the newborns blood is still unknown. But a consequence is an increased permeability of the red cell membrane (15) which could facilitate the transport of many compounds across the membrane.

### SUMMARY

Methods have been adapted and their reproducibility tested for the determination of blood lipids and the fatty acid composition of the phosphoglycerides. The main lipid classes in 20 women from the south western part of Sweden at term pregnancy and of umbilical cord plasma of their newborn infants were studied. Determinations were made of the fatty acid pattern of plasma phosphoglycerides and of cephalins and lecithin of red cells.

The concentration of the major lipid classes in maternal and umbilical cord plasma agreed well with previously reported figures from other countries. The maternal plasma fatty acid pattern of phosphoglycerides was similar to that of adult males. The mothers and their infants had identical concentrations of the total sum of all polyunsaturated fatty acids in plasma phosphoglycerides in red cell lecithin and in red

cell cephalins. The infants pattern differed from the mothers by much lower concentrations of the parent fatty acids linoleic and linolenic acids and correspondingly increased concentrations of the more polyunsaturated fatty acids of the two series. The fatty acid composition in the red cell lecithin of mother and infant reflected that in the corresponding plasma. The cephalins contained more polyunsaturated fatty acids than the lecithin in both mothers and infants red cells.

The study produced no biochemical evidence of an essential fatty acid deficiency in these newborns. The identical concentration of total polyunsaturated fatty acids in blood phosphoglycerides of mothers and infants in combination with the pronounced change in the ratio between the parent fatty acids and the highly polyunsaturated derivatives suggest a homeostatic regulation of the fatty acid composition of the blood phosphoglycerides.

### ACKNOWLEDGEMENTS

We are greatly indebted to Mrs Gunilla Carlgren for expert help with the dietary records. The skilful technical assistance of Miss Anna Björklund is gratefully acknowledged. The authors are grateful to the Swedish Medical Research Council for the statistical service in Göteborg.

This study was supported by grants from the National Association for Research on Heart and Lung Diseases, the Association for the Aid of Crippled Children, New York, the C. B. Nashborn Scientific Foundation and Euphrates' Fund for Prenatal Research.

### REFERENCES

1. Alling C, Dencker S J, Brennerholm L & Tichy J. Serum fatty acid pattern in chronic alcoholics after acute abuse. *Acta Med Scand* 183: 99 1969.
2. Barnes R H, Robertson Cumuld S, Zimmerman R, Summers H, MacLeod R B & Krock L. Influence of nutritional deprivations in early life on learning behavior of rats as measured by performance in a water maze. *J Nutr* 89: 399 1966.
3. Bartlett G R. Phospholipid assay in column chromatography. *J Biol Chem* 234: 466 1959.
4. Beroohn J & Waybome S. Serum cholesterol concentration in newborn african and european

newborns than in the mothers red cell cephalins 50% and 48% respectively

There are few reports on the fatty acid composition of red cell cephalins in the literature (16, 17, 55-56). The present figures for the fatty acid composition of mothers red cell cephalins show a very good agreement with the figures of Dodge & Phillips (16) for ethanolamine and serine phosphoglycerides of male adult red cells. In our study 16:0 was larger, 20:4 ( $n=6$ ) smaller and 22:6 ( $n=3$ ) larger than in their study but the differences were small. The fatty acid composition of the newborns red cell cephalins in this study is very similar to that found by Crowley et al. (12) for newborns ethanolamine plus serine phosphoglycerides.

**Mother-infant relation.** The sum of all fatty acids of the linoleic acid series in plasma phosphoglycerides and red cell lecithin in the infants blood was only slightly lower than in the mothers blood and the differences were significant only at the 0.01 level. In the cephalins this sum was about 25% higher in the newborns than in the mothers; the difference significant at the 0.001 level. The concentration of linoleic acid, 18:2 ( $n=6$ ) was much lower in both plasma and red blood cells of newborns blood than of maternal blood (Tables 5-6). The lower 18:2 concentration of the cord blood was associated with much higher concentrations of the more unsaturated fatty acids of the linoleic acid series, mainly 20:4 ( $n=6$ ) and 20:3 ( $n=6$ ). These findings are in complete agreement with previous reports (44, 50, 58). A correlation was found between the concentrations of linoleic acid in maternal and cord plasma phosphoglycerides significant at the 0.05 level ( $r=0.478$ ,  $n=20$ ).

The concentration of fatty acids of the linolenic acid 18:3 ( $n=3$ ) series was higher in cord blood plasma phosphoglycerides and red cell lecithin than in the mothers blood. By contrast in the cephalins the sum of the fatty acids of the linolenic acid series was significantly higher in the mothers red cells than in the infants (Table 6). The pattern of the fatty acids of this

series showed the same tendency as the linoleic acid series. The level of the parent fatty acid 18:3 ( $n=3$ ) was lower in the infants than in the mothers plasma phosphoglycerides and red cell lecithin, but the more polyunsaturated acids were increased. The figures for 18:3 ( $n=3$ ) are, however, uncertain because 18:3 ( $n=3$ ) was not separated from 20:1 with the polar column used for GLC separation of fatty acids.

The present study demonstrated very small individual variations in the blood phosphoglyceride fatty acid pattern of mothers in labour and their infants. The small variations support the view that environmental factors play at most a minor role compared to a homeostatic mechanism for the regulation of the fatty acid pattern of blood phosphoglycerides. The influence of dietary factors on plasma lipids and fatty acids of mothers and their infants was studied by Hansen et al. (25). They found no correlation between mother's intake of linoleic acid, varying from 1.5 to 8.6% of calories and the serum dienoic and tetraenoic acid levels of her infant at birth. Renkonen (44) found substantially lower concentrations of the fatty acids of the linoleic acid series in plasma phosphoglycerides of Finnish mothers and their infants. He ascribed the low levels of fatty acids of the linoleic acid series to the large proportion of milk fats poor in linoleic acid in the Finnish diet. A low recovery of all the polyunsaturated fatty acids better explains his results.

In essential fatty acid deficiency 20:3 ( $n=9$ ) is increased (18, 28, 37). The concentration of this acid was very low in our normal adults but the level was significantly higher in the newborns blood, and a slight relative lack of essential fatty acids of the newborns cannot be excluded. The most polyunsaturated fatty acids of the linoleic and linolenic acid series were however much higher in the infants than in the mothers blood and the increase of 20:3 ( $n=9$ ) of the oleic acid series might only reflect a generalized increased desaturation and chain elongation of the fatty acids also in this series.

The inverted 18:2/20:4 ratio of the lecithin and cephalins in newborns blood com-

- composition of sphingomyelin and lecithin in normal human serum. *Proc Soc Exp Biol Med* 119 347 1965
1. Prutchin S N, Cameron A H, Davidson S, Dean P M, Foadbrooke A S, Lloyd J K, Miles J M & Wolff O H. The diabetic pregnancy. A study of serum lipids in maternal and umbilical cord blood and of the uterine and placental vasculature. *Arch Dis Child* 39 334 1964
  2. Phillips G B & Dodge I T. Composition of phospholipids and of phospholipid fatty acids of human plasma. *J Lipid Res* 8 676 1967
  3. Poli L & Berni G. Metodologia nuova per lo studio dei lipidi ematici. Valori medi nella madre e nel feto. *Pediatrics ucrnales* (Rome) 3 267 1955
  4. Ralstedt S. Studies on serum lipids and lipoproteins in infancy and childhood. *Acta Paediatr Scand* 44 Suppl 102 1955
  5. Renkonen O V. Serum lipids of labouring mothers and newborn babies. *Ann Med Exp Biol Fenn* 44 Suppl 10 1966
  6. Riss E M, Eden H A & Bass O P. Protein-lipid relationships in human plasma. III. In pregnancy and the newborn. *J Clin Invest* 33 1667 1954
  7. Schrade W, Bobbe E, Baegler R, Tacke R & Ulrich B. Geschromatographische Untersuchungen der Serumfett-säuren des Menschen. III. Die Fettsäuren der Cholesterinester, Phospholipide und Triglyceride sowie die unveresterten Fettsäuren bei Gesunden und bei Arteriosklerotikern. *Klin Wochr* 38 739 1960
  8. Schwartz O H, Soule S D & Dumas B. Blood lipids in pregnancy. *Amer J Obstet Gynec* 39 203 1940
  9. Seidewitz G W. *Statistical methods*. Iowa State College Press Ames Ia 1957
  10. Sobor E, Bosnak E T, Wang C I & Adlersberg D. Serum composition in the newborn. *Science* 123 461 1956
  11. Sprecher H & Robertson A. Analysis of lecithin and sphingolipid fatty acids from human adult and fetal blood perfused placenta chorion laeve and amnion. *J Lab Clin Med* 70 489 1967
  12. Svannberg A & Svennerholm L. Plasma total lipid, cholesterol, triglycerides, phospholipids and free fatty acids in a healthy Scandinavian population. *Acta Med Scand* 169 43 1961
  13. Svannholm L. Isolation of gangliosides. *Acta Chem Scand* 17 239 1943
  14. —. Distribution and fatty acid composition of phosphoglycerides in normal human brain. *J Lipid Res* 9 570 1968
  15. Sweeney M J, Erteldorf J N, Dobbins W T, Somerville B, Fischer R & Fenell C. Dietary fat and concentrations of lipid in the serum during the first six to eight weeks of life. *Pediatrics* 27 765 1961
  16. Ways P & Hanahan D J. Characterization and quantification of red cell lipids in normal man. *J Lipid Res* 5 318 1964
  17. Williams I H, Wachmak M & Witter R. F. Fatty acids in phospholipids isolated from human red cells. *Lipids* 1 391 1966
  18. Woodruff C W, Barley M C, Davis J T, Rogers N & Coniglio J G. Serum lipids in breast fed infants and in infants fed evaporated milk. *Amer J Clin Nutr* 14 84 1964
  19. Zee P. Lipid metabolism in the newborn. I. Phospholipids in cord and maternal sera. *Pediatr res* 39 82 1967
  20. Zoffner N, Wolfrum G, Loozong W & Jurach K. Untersuchungen über die Plasma lipide des Neugeborenen, Säuglings und Kleinkindes. *Klin Wochr* 44 380 1966

Submitted Febr 16 1970

Accepted March 26 1970

(R. O.) Dept of Paediatrics  
University of Göteborg  
Göteborg  
Sweden

**Key words** Gas liquid chromatography, plasma, red cells, phosphoglycerides, fullterm infants, mothers in labour



- infants and their mothers *Amer J Clin Nutr* 4 117 1956
- 5 Boyd E M Lipid composition of blood in new born infants *Amer J Dis Child* 52 1319 1936
- 6 Brody S & Carlsson L A Plasma lipid concentrations of the newborn with special reference to the distribution of the different lipid fractions *Clin Chim Acta* 7 694 1962
- 7 Brown D F McCandly R B Gillie E & Doyle T Observations on some serum components in mothers and in their newborn infants *Amer J Obstet Gynec* 77 536 1969
- 8 Carlsson L A Determination of serum glycogen *Acta Soc Med Upsal* 64 208 1959
- 9 Cramér K & Vikrol O Plasma phospholipid levels and distribution in human maternal blood and in blood from the umbilical cord *Acta Med Scand* 179 1 1966
- 10 Cravioto J De Licardie E R & Bitch H G Nutrition growth and neurointegrative development An experimental and ecologic study *Pe diatrict* 38 319 1966
- 11 Crawford N An improved method for the determination of free and total cholesterol using the ferric chloride reaction *Clin Chim Acta* 3 357 1958
- 12 Crowley J Ways P & Jones J W Human fetal erythrocyte and plasma lipids *J Clin Invest* 44 989 1965
- 13 Dannenburg W A Burt R L & Leske N H Plasma lipids in the early puerperium *Amer J Obstet Gynec* 84 1091 1962
- 14 Dannenburg W A & Burt R L The effect of insulin and glucose on plasma lipids during pregnancy and the puerperium *Amer J Obstet Gynec* 92 195 1965
- 15 van Deenen L L M Phospholipids and bio membranes *Progress in the Chemistry of Fats and other Lipids* 8 1965
- 16 Dodge J T & Phillips G B Composition of phospholipids and of phospholipid fatty acids and aldehydes in human red cells *J Lipid Res* 8 667 1967
- 17 Farquhar J W & Ahrens E H Effects of dietary fat on human erythrocyte fatty acid pattern *J Clin Invest* 42 675 1963
- 18 Fukuo A J & Mead J F Metabolism of essential fatty acids VIII Origin of 5, 8, 11 eicosatrienoic acid in the fat deficient rat *J Biol Chem* 234 1451 1959
- 19 Gordon M B & Cohn D J Cholesterol and lipid phosphorus in infancy and in childhood *Amer J Dis Child* 35 193 1928
- 20 Graven S N Manning G Sagerman R & Rikkers H A study of phospholipids in neonatal hyperbilirubinemia *J Pediatr* 66 12 1965
- 21 Gyorgy P Über den Lipidgehalt des Nabelschnur Blutserums und des mütterlichen Blutes *Klin Wochr* 3 483 1924
- 22 Hadnagy C S Lonney E A Takács T Boaru R P Kisor I Erdélyi A Pappá I & Róna M Lipid contents of the newborn's blood *Biol Neonat* 5 390 1963
- 23 Hagerman D D & Vilee C A Transport functions of the placenta *Physiol Rev* 40 313 1960
- 24 Hallgren H Stenbom S Svanborg A & Svennerholm L Gaschromatographic analysis of the fatty acid composition of the plasma lipids in normal and diabetic subjects *J Clin Invest* 39 1424 1960
- 25 Hansen A F Wiese H F Adam D J D Boettche A N Haggard M E Davis H Newson W T & Petat I Influence of the blood serum lipids in pregnant women and newborn infants *Amer J Clin Nutr* 15 11 1964
- 26 Herman E & Neumann J Über den Lipidgehalt des Blutes normaler und schwangerer Frauen sowie neugeborener Kinder *Biochem Z* 43 41 1912
- 27 Hershfield M & Neweth A M Plasma FFA in mother and fetus Placental transport of palmitic and linoleic acid in the guinea pig *Fed Proc* 26 2 846 1967
- 28 Holman R T Caster W D & Wiese H F The essential fatty acid requirement of infants and the assessment of their dietary intake of linoleate by serum fatty acid analysis *Amer J Clin Nutr* 14 70 1964
- 29 Hornung H Die osmotische Resistenz der Erythrocyten und der Lipidgehalt des Blutes bei Mutter und Kind *Deutsch Med Wochr* 44 1849 1926
- 30 Jacino J Komoros M Mathéford E & Teckler V Die Gesamtlipide der Lipiden Phosphor des freien und esterifizierten Cholesterols im Serum der Mutter und im Nabelschnurblut *Zbl Gynec* 23 902 1961
- 31 Jatzkowitz H & Mehl E Zur Dünndarmchromatographie der Gehirn Lipide überer Um und Abbauprodukte *Hoppe Seyler Z Physiol Chem* 330 251 1960
- 32 Kaplan A & Lee V F Serum lipid levels in infants and mothers at parturition *Clin Chim Acta* 12 258 1965
- 33 Meeberg J Lipid determinations in maternal and foetal blood *J Obstet Gynec Brit Comm* 76 701 1963
- 34 Kontinen A & Pyörälä T Serum lipid patterns in normal pregnancy and pre-eclampsia *J Obstet Gynec Brit Comm* 71 453 1964
- 35 Mender J Savits B S Flores M & Sarmahar N S Cholesterol levels of maternal and fetal blood at parturition in upper and lower income groups in Guatemala City *Amer J Clin Nutr* 7 595 1969
- 36 Mitscher A Über Steroide 49 Mitteilung Über Farbreaktionen *Helv Chim Acta* 29 743 1946
- 37 Mohrbauer H & Holman R T The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver *J Lipid Res* 4 151 1969
- 38 Mortimer J C Cord blood lipids of normal infants and infants of diabetic mothers *Arch Dis Child* 39 334 1964
- 39 O'Brien J S & Blankenhorn P H Fatty acid

Table 1 Regression equations of vital capacity and expiratory flow rates against height

Dependent variable	Equation	r
FVC vs. spirometer (litre)		
Male	$0.113 \pm 0.02111$ ht	0.925
Female	$0.145 \pm 0.01986$ ht	0.942
FVC wedge spirometer (litre)		
Male	$0.124 \pm 0.02072$ ht	0.909
Female	$0.153 \pm 0.01944$ ht	0.936
MMEF water spirometer (litre/sec)		
Male	$0.06063$ ht - 5.9353	0.739
Female	$0.05247$ ht - 4.8715	0.770
$\dot{V}_{max}$ wedge spirometer (litre/sec)		
Male	$0.06913$ ht - 6.8291	0.754
Female	$0.05648$ ht - 4.96764	0.669
EFPR Wright peak flow meter (litre/min)		
Male	$0.09530$ ht - 8.40512	0.878
Female	$0.10100$ ht - 9.20783	0.866
$\dot{V}_{max}$ wedge spirometer (litre/sec)		
Male	$0.08024$ ht - 6.6184	0.796
Female	$0.08278$ ht - 7.02453	0.811

## RESULTS AND DISCUSSION

## 1 Normal children

The regression equations for the forced vital capacity and flow rates plotted against standing height performed with the wedge and water spirometer are given in Table 1.

The FVC and the FEV<sub>1</sub> measured by the two instruments were not significantly different (Figs 1 and 2).

$\dot{V}_{max}$  with the wedge spirometer was found to be significantly higher than the MMEF when performed with the water spirometer ( $p < 0.001$ ). However the MMEF using the wedge spirometer and recorded with the UV recorder on the same breath as the  $\dot{V}_{max}$  was the same as MMEF using the water spirometer. There was a good correlation between  $\dot{V}_{max}$  and height. This is also true for MMEF (Table 1).

Figs 3 and 4 show the relationship between  $\dot{V}_{max}$  and MMEF and the degree of correction required between these two flow rates.

The  $\dot{V}_{max}$  measured with the wedge and the

three distinct flow volume curves stored on the oscilloscope screen was taken with an oscilloscope camera with adaptor. For calculation the EFVC with the highest VC was recorded. Peak expiratory flow ( $\dot{V}_{max}$ ) and the maximal flow rate at the 50% point of the FVC ( $\dot{V}_{50}$ ) on the flow volume curve were calculated.

The FEV marker was initially set to start the one second interval when the flow exceeded 40 ml/sec. Some of the subjects pretriggered the marker generator by emphyseal breath holding. This caused a discrepancy between the FEV on the flow volume curve and the FEV calculated from the UV recorder on the same breath. Decreasing the sensitivity of the marker generator to 100 ml/sec eliminated this discrepancy without significantly changing the FEV.

In order to assure experimental consistency all instruments were calibrated daily. The oscilloscope was calibrated in both the abscissa and the ordinate by means of an internal calibration signal. For calibration of the wedge and the UV recorder we used a graduated 1 litre syringe for volume and a rotameter calibrated at 1 litre/sec. for flow.

The expiratory peak flow rate (EFPR) was recorded on Wright's peak flow meter with the subject standing and repeating the performance until the highest possible flow rate was obtained.

Model 197A Hewlett Packard Co. Colorado Springs, Colo. USA.

Model 10A1077A Fisher Porter.

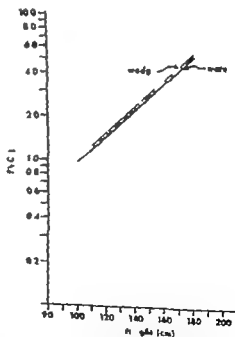


Fig. 1 A comparison in healthy children between FVC measured with the water spirometer (FVC = 0.113 ht - 0.118) (solid line) and the wedge spirometer FVC = 0.125 ht - 0.1215 (dashed line).

## EXPIRATORY FLOW RATES DETERMINED BY WEDGE AND WATER SPIROMETER IN CHILDREN AND YOUNG ADULTS

H LEVISON M KAMEL T R WENG and K KRUGER

*From the Research Institute of the Hospital for Sick Children and the Department of  
Paediatrics University of Toronto Toronto Ontario Canada*

Pulmonary function tests are being used with increasing frequency in the investigation and diagnosis of pulmonary disease in children. One of the commonest of the routine pulmonary function tests is the forced vital capacity (FVC) manoeuvre. The flow volume technique was first described by Hyatt et al. (4) and the theory of the relationship of flow, volume and trans pulmonary pressure was later elaborated by these same authors (2, 3) thus providing the basis for the study of the expiratory flow volume curve (EFVC).

It has been suggested (7) that the EFVC as measured by the waterless wedge spirometer will replace the more common FVC manoeuvre as performed with the standard water spirometer as a routine pulmonary function test.

Normal values for children have been reported for the parameters of the FVC test using the standard water spirometer (5, 8). There have been no normal values published for the EFVC using the wedge spirometer. Measurements of the parameters of the EFVC have been published for children using a volume displacement plethysmograph (9) and by a method

which incorporates a pneumotachograph and a light weight spirometer (6). These latter two techniques require sophisticated equipment. The wedge spirometer is simple in design and requires little training in its use.

The purpose of the study was to compare flow measurements obtained using a wedge spirometer with those obtained using a water spirometer in both healthy and asthmatic children and young adults.

### MATERIALS AND METHOD

Eighty-two subjects (40 males, 42 females) aged 6 to 18 years ranging in height from 111.5 cm to 176.5 cm and with no history of cardio-pulmonary disease were studied. Fourteen symptomatic asthmatics (5 males, 9 females) aged 8 to 15 years were also studied.

Water spirometry was performed with a standard 9 litre water spirometer. EFVC, forced expiratory volume in 1 sec (FEV<sub>1</sub>) and maximal mid expiratory flow (MMEF) were measured while the subject performed a forced vital capacity manoeuvre. Each manoeuvre was performed with the subject standing and was repeated three times; the record with the highest value for FVC being selected.

Wedge spirometry was performed with a "70 wedge spirometer". The standing subject was connected by a large diameter tube to the perimeter with the electrical outputs connected to an oscilloscope and an ultra violet recorder (UV recorder). A flow triggered one second marker generator developed in this laboratory was connected to the oscilloscope so that the flow volume curve was displaced by 1 cm perpendicular to the volume axis for a 10 ml/sec interval. 1 sec after initiation of flow. As the subject repeatedly performed the forced vital capacity manoeuvre a picture of

Supported by a grant from the Canadian Cystic Fibrosis Foundation.

• Warren & Collins Braintree Mass. USA  
• Mod 1370 Med Science Electronics St. Louis Mo. USA  
• Model 564B Tektronix Inc. Beaverton Ore. USA

• S E Laboratories Ltd. Feltham Middlesex England

Table 2 Comparison of  $V_{50}$  and  $\dot{V}_{max}$  values in normal and previously reported studies

Study	Subject's height 130 cm		Subject's height 170 cm	
	$V_{50}$ (litres/ sec)	$\dot{V}_{max}$ (litres/ sec)	$V_{50}$ (litres/ sec)	$\dot{V}_{max}$ (litres/ sec)
Zapletal et al (9)				
Male	2.48	3.49	4.65	6.72
Female	2.45	3.19	4.25	5.83
DeMuth et al				
Male	1.75	3.07	2.82	6.42
Female	1.79	2.67	2.96	5.77
Present study				
Male	2.16	3.81	4.92	7.02
Female	2.37	3.74	4.63	7.05

comparable to the FVC measured by the water spirometer. In normal subjects we found the  $\dot{V}_{50}$  to be greater than MMEF and in asthma not to be less. This cannot be accounted for by a change in lung volume since the vital capacity was unchanged suggesting that  $\dot{V}_{50}$  is a more sensitive index of airway obstruction than MMEF. The fact that the  $\dot{V}_{50}$  is consistently higher than the MMEF in normal children is due not to the instrument in question, but to the fact that the MMEF is a mean flow over a wide range whereas  $\dot{V}_{50}$  is an instantaneous measure of flow.

This finding in symptomatic asthmatic patients agrees with those of Stott et al (7) in adult patients with chronic obstructive pulmonary disease.

The EFVC technique using the wedge spirometer is as simple to perform as the FVC manoeuvre with the water spirometer and easier to calculate also the shape of the curve obtained using the wedge spirometer may be in itself diagnostic of obstructive or restrictive lung disease (6). Another advantage of the EFVC is that it includes a measurement of peak flow and can be modified to show a time volume relationship. However the equipment is expensive and its electrical calibration must be checked for both flow and volume to ensure consistent results.

A comparison of the  $\dot{V}_{50}$  and  $\dot{V}_{max}$  predicted for heights of 130 and 170 cm respectively for the present study and those of Zapletal et al (9) and De Muth et al (1) are given in Table 2. The values of  $\dot{V}_{50}$  predicted for the present study are much closer to Zapletal's than DeMuth's but the results for  $\dot{V}_{max}$  in the three studies are reasonably close. The main differences between the three studies are probably due to differences in methods.

## SUMMARY

A comparison between measurements made with a conventional water spirometer and those with a wedge spirometer on 82 healthy normal subjects from 6 to 18 years of age and from 111.5 to 176.5 cm in height was made. Fourteen children with symptomatic asthma were also studied. It was found that the FVC and FEV<sub>1</sub> were similar on both instruments. The values for  $\dot{V}_{50}$  and MMEF were different because  $\dot{V}_{50}$  is an instantaneous measurement whereas MMEF is a mean obtained over a wide range of lung volumes.

It is suggested that  $\dot{V}_{50}$  may be a more sensitive index of airway obstruction than the regular MMEF measurement.

## REFERENCES

- DeMuth G R, Howatt W F & Hill B M. The growth of lung function. *Pediatrics* 35 Suppl 161 1965.
- Fry D L. Theoretical considerations of the bronchial pressure-flow-volume relationships with particular reference to the maximum expiratory flow-volume curve. *Phys Med Biol* 3 174 1958.
- Fry D L & Hyatt R E. Pulmonary mechanics. A unified analysis of the relationship between pressure, volume and airflow in the lungs of normal and diseased human subjects. *Amer J Med* 29 672 1960.
- Hyatt R E, Schilder D P & Fry D L. Relationship between maximum expiratory flow and degree of lung inflation. *J Appl Physiol* 13 331 1958.
- Kamel M, Weng T R, Featherby E A, Jackson W S & Lerman H. Relationship of mechanical ventilation to lung volumes in children and young adults. *Scand J Resp Dis* 50 125 1969.

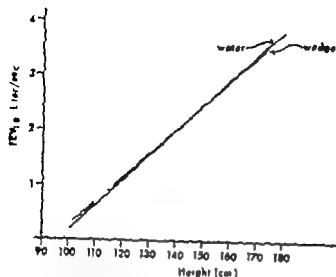


Fig 2 A comparison in healthy children between  $FEV_1$  measured with the water spirometer ( $FEV_1 = 4.368 + 0.0457 \times ht$ ) solid line and the wedge spirometer ( $FEV_1 = 4.199 + 0.0445 \times ht$ ) dashed line

EPFR measured with the Wright peak flow meter were not significantly different ( $0.6 > p > 0.5$  paired  $t$ )

## 2 Asthmatic children

When  $V_{50}$  was plotted against MMEF for the 14 asthmatic patients the regression line was

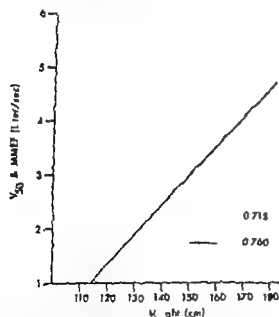


Fig 3 Regression in healthy children of MMEF measured with the water spirometer ( $MMEF = 0.057 \times ht - 5.43$   $r = 0.76$ ) solid line plotted against height and that of  $V_{50}$  measured with the wedge spirometer ( $V_{50} = 0.063 \times ht - 5.942$   $r = 0.713$ ) dashed line plotted against height

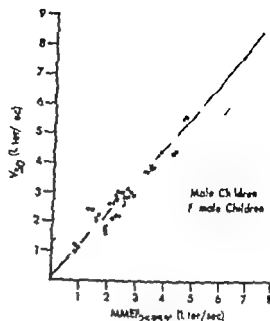


Fig 4 The relationship in healthy children between MMEF measured with the water spirometer and  $V_{50}$  measured with the wedge spirometer ( $V_{50} = 1.143 \times MMEF + 0.035$ ) Mean regression line with 95% confidence limits is shown

calculated to be  $V_{50} = 1.11 \times MMEF - 0.16$  ( $r = 0.81$ ) (Fig 5). This regression line gives a lower value for  $V_{50}$  for the corresponding MMEF when compared to the regression line for the healthy children  $V_{50} = 1.143 \times MMEF + 0.035$  ( $r = 0.95$ ).

From the above results it is clear that the FVC as measured by the wedge spirometer is

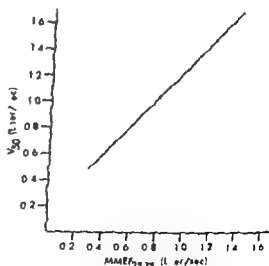


Fig 5 Solid line represents the regression line of  $V_{50}$  plotted against MMEF in normal children the dashed line represents the regression line of  $V_{50}$  plotted against MMEF for the 14 symptomatic asthmatics

## RESPIRATORY QUOTIENT AND METABOLIC RATE IN NORMAL FULL TERM AND SMALL FOR DATE NEWBORN INFANTS<sup>1</sup>

J SENTERRE and P KARLBERG

*From the Department of Paediatrics University of Göteborg Sweden*

The reports concerning the respiratory quotient (RQ) during the first week of life generally agree that the RQ begins to fall shortly after birth but rises again a few days later (4 7 15 16 31). Recent studies have shown a rise in the level of free fatty acids (FFA) glycerol and ketone bodies in the blood of newborn infants during the first few hours after birth (2 11 23 26 28 32 36). Likewise the amount of FFA and the amount and rate of release of glycerol in the newborn's adipose tissue are higher on the first postnatal day (33). On the other hand at the same time the carbohydrate reserve and the blood glucose level decrease (6 39 40). Thus endogenous fat seems to be mobilized after birth indicating increased use of lipids for energy production.

Some newborn infants have reduced (premature small for date) or greater (babies of diabetic mothers) carbohydrate and fat reserves. After birth the blood level of glycerol and ketone bodies rises rapidly to higher values in premature and small for-date infants than in full term ones (27).

We have thought that a study of postnatal changes in the RQ of small for-date and normal newborn babies might also show from

another view point possible differences in energy metabolism.

### MATERIAL AND METHODS

#### *Material*

Eight normal and full term and six small for-date newborn infants were studied several times from delivery to the end of the first week of life. The normal full term babies had a mean birth weight of 3.4 kg and a gestational age between 39 and 42.5 weeks. The small for-date infants had a mean birth weight of 2 kg and a gestational age between 36 and 40 weeks. When plotted on the Engstrom & Serris weight gestational age chart (12) all of the small for-date babies fell below the tenth percentile line (Fig. 1).

#### *Methods*

Respiratory metabolism was measured by an open circuit method using the kipp diaferometer. The baby was enclosed in a double Perspex box (22). The air which flowed between the two perspex layers toward the foot end was pumped at a known constant rate (3.83 or 4 l/min) through the gas analyzer. The chamber was placed inside an Isolette incubator. Both oxygen uptake and carbon dioxide production were measured and recorded by a kipp channel selector and a potentiometric Micrograph. Each determination corresponds to 3 hours of recording. Activity was continuously monitored by constant observation and graded by using the arbitrary scale of Oliver & Karlberg (34). Only score 0 infant asleep and not moving or score 1 infant asleep moving one extremity or baby with open eyes but physically quiet were accepted for determination of the RQ and the basal oxygen consumption. The babies were usually examined between two meals and received breast milk after 12 hours of life. No drugs were given.

Temperatures were measured and recorded (Pin tip) by multiple thermocouples. These were rectally placed on the abdominal skin in the air stream above the baby's feet at the head-end near the exhaust

<sup>1</sup>Aided by grants from the Belgian University Fund the Belgian Scientific Research Fund the Swedish Medical Research Council grant B47 19X 17403 and the Association for the Aid of Crippled Children New York.

*Present address:* Pediatric Department State University of Liège Belgium.

- 6 Lord G P, Gazdaglu K & Kallreider N. The maximum expiratory flow volume in the evaluation of patients with lung disease. A comparative study with standard pulmonary function tests. *Amer J Med* 46: 72, 1969.
  - 7 Stout, B D, Wiener L, Dery B W & Cox J W. Clinical application of the flow volume loop. *Dis Chest* 55: 101, 1969.
  - 8 Weng T R & Levison H. Standards of pulmonary function in children. *Amer Rev Resp Dis* 99: 879, 1969.
  - 9 Zapletal A, Motoyama E K, Van De Woestijne K P, Hunt V R & Bouhuys A. Maximum expiratory flow volume curves and airway conductance in children and adolescents. *J Appl Physiol* 26: 308, 1969.
- Submitted Jan 14, 1970  
Accepted May 23, 1970
- (H. L.) The Research Institute  
The Hospital for Sick Children  
555 University Avenue  
Toronto 101, Ontario, Canada
- Key words:** Wedge, water spirometer, respiratory flow rates.

## DISCUSSION

Protein metabolism apparently contributes little to the energy requirement during the early neonatal period. McCance & Strangeways have shown that protein catabolism provides less than 4% of the energy during the first 48 hours of life, whereas in adults basal calories are derived up to about 17% from protein catabolism (25). Thus a fall of the RQ values shows acceptable evidence of fat utilization being substituted for carbohydrate utilization as a source of calories. However the RQ value in the newborn infant must be cautiously interpreted.

First a difference between carbon dioxide output and production may exist over a short period of time (13). From continuous records during several hours wide variations of the RQ have been observed in the present material. The RQ values are high in a period of activity and fall steeply in the early ensuing sleep (Fig. 5). This could be explained chiefly by unsteady ventilation with drop of the blood  $\text{CO}_2$ -tension during activity and a recovery during sleep. As a significant increase of the blood lactate has been observed in newborns after a period of activity (14) an oxygen debt could explain low RQ values during the recovery period. High RQ values in muscular activity might also be a consequence of increased carbohydrate catabolism in the muscles.

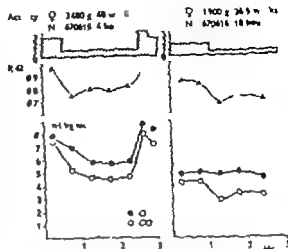


Fig. 5  $\text{CO}_2$  production,  $\text{O}_2$  uptake and RQ values observed in a period of activity (2 or 3) and in the following quiet period (0 or 1) in normal (left) and small for date (right) newborn babies. Observe a steady  $\text{O}_2$  consumption in the small for-date infant.

After half an hour of sleep a steady state of RQ was usually achieved. However rather often slight variations of  $\text{CO}_2$  production have been noticed without significant changes in oxygen consumption. Sometimes the  $\text{CO}_2$  production tends to decrease progressively and then suddenly rises to a higher and new steady state value. This is likely related to eye movements and modification of the ventilation during sleep (10, 35). Secondly the RQ falls with increasing oxygen consumption in response to cold (5, 8, 21, 22, 34, 42). During the observation periods the infant was maintained in a neutral range of environmental temperature. Moreover as the radiant heat loss from the skin to the cold walls of the incubator is at least as important as the convective heat loss (30) such losses were prevented by controlling the ambient temperature and by using a double perspex chamber (16). The temperature gradient on both sides of the chamber is less than 1°C, this condition preventing radiant heat loss.

A systematic trend of the RQ values in the healthy newborn infant has been described in previous studies (4, 7, 20, 31).

The RQ falls during the first days, rises again during the next few days to about 0.86.

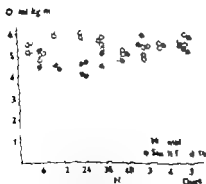


Fig. 4 Values of oxygen consumption in small for date and normal birth weight infants during the first 5 days of life.



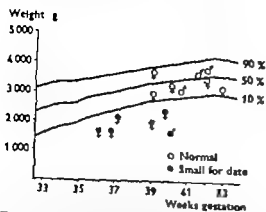


Fig 1 Birth weights of normal and small-for-date babies are plotted in regard to number of weeks gestation. The percentile ranges for female newborn infants according to Engstrom & Sterky (12)

tube and inside the incubator. The temperature at the head end was considered to be the environmental temperature and was maintained within the neutral range according to the Scope chart (37).

Stool and urinary output were carefully observed. As a matter of fact under our technical conditions the oxygen deflection dropped sharply when the chamber within the incubator was contaminated by a few drops of urine. This is probably due to the evaporation of the ammonia influencing the gas analyser. When such effects were observed the chamber was cleaned and the measurement repeated.

## RESULTS

The RQ values in both groups of newborn babies are plotted with regard to the age in Fig 2. From the statistical analysis of the data two regression lines have been drawn for each group. In normal birth weight babies the RQ decreases ( $r = -0.63$ ) significantly ( $p <$

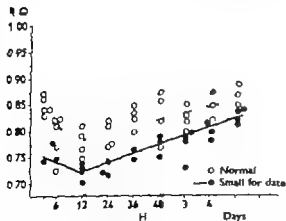


Fig 2 RQ values in both groups of newborn babies are plotted in relation to the age. Two regression lines have been drawn for each group one up to 12 hours of age and the other from 12 hours to 5 days.

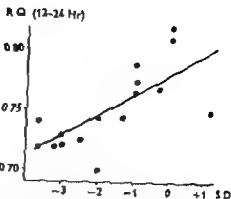


Fig 3 Relationship between the lowest RQ value observed between 12 and 24 hours of life in both groups of newborn babies. The differences between actual and predicted weights are expressed in standard deviation units from the mean values according to the intrauterine growth chart.

0.05) from the third to the twelfth hour of age. From the twelfth hour to the fifth day the RQ values increase ( $r = +0.56$ ) significantly ( $p < 0.01$ ) up to a value of 0.87. For the small for date babies the RQ values in the first 12 hours are significantly lower ( $p < 0.001$ ) than in normal birth weight babies and a similar drop is observed. From the twelfth hour the RQ values increase ( $r = +0.81$ ) significantly ( $p < 0.001$ ) the regression line is lower ( $p < 0.001$ ) and its slope is steeper ( $p < 0.001$ ) than in the control group. The RQ values tend to be similar in both groups as at the end of the first week of life.

In order to investigate a possible relationship between the degree of small for date and the lowest RQ values, the latter are plotted with regard to the differences in standard deviation units between the actual and the predicted mean values of birth weight according to the birth weight gestational age (12) (Fig 3). The more pronounced the smallness for date, the less was the RQ at the twelfth to twenty-fourth hour of life. Although the values are scattered the slope of the regression line is statistically significant ( $p < 0.001$ ) and the coefficient of correlation is 0.72. The mean value of oxygen consumption in small for date infants ( $4.8 \pm 0.5$  ml/kg/min) is significantly lower ( $p < 0.001$ ) than the normal full term mean value ( $5.4 \pm 0.4$  ml/kg/min) (Fig 4).

and rose again during the next few days. However, the RQ was lower during the first 5 days in small for date infants. The O<sub>2</sub> uptake of the small for date babies is initially lower but rises during the first 5 days.

Technical problems and clinical implications of the results are discussed.

## REFERENCES

- Adams G. A., Gandy G. M. & James L. S. The influence of thermal factors upon oxygen consumption in the newborn human infant. *J. Pediatr.* 66: 495, 1965.
- Abernethy H., Abom T. & Somersalo O. Acid base balance in blood of infants and children of various ages. *Ann. Paediat. Fenn.* 11: 108, 1965.
- Auld P. A. M., Brongnioude P. & Mehta S. The influence of an early caloric intake with IV glucose on catabolism of premature infants. *Pediatrics* 37: 592, 1966.
- Benedict F. G. & Talbot F. B. *The physiology of the newborn infant. Character and amount of the metabolism.* Publ. 233. Carnegie Institution of Washington, 1915.
- Brock K. Temperature regulation in the newborn infants. *Biol. Neonat.* 5: 65, 1961.
- Corabath M., Segal S. & Smith C. A. Carbohydrate and energy metabolism in the newborn. An international exploration. *Pediatrics* 39: 582, 1967.
- Cross K. W., Tizard J. P. M. & Tryballe D. A. H. The gaseous metabolism of the newborn infant. *Acta Paediat. Scand.* 46: 265, 1957.
- Dawkins M. J. R. & Scopes J. W. Non shivering thermogenesis and brown adipose tissue in the known newborn infant. *Nature* 206: 201, 1965.
- Dickerson R. A., Wilkinson R. H., Davies P. A. & Asenworth P. Plasma glucose levels in infants weighing 500 g and less fed immediately after birth with breast milk. *Biol. Neonat.* 11: 29, 1967.
- Dreyfus-Brown C. & Monod N. Sleep of premature and full-term neonates. A polygraphic study. *Proc. Roy. Soc. Med.* 58: 6, 1965.
- Van Deyne C. M. & Havel R. J. Plasma unesterified fatty acid concentration in foetal and neonatal life. *Proc. Soc. Exp. Biol. Med.* 102: 599, 1959.
- Engstrom L. & Sterky G. Standardkurvor for vikt och längd hos nyfödda barn. *Läkertidningen* 61: 49—, 1966.
- Fhari L. E. & Rahm N. Gas stores of the body and the unsteady state. *J. Appl. Physiol.* 7: 472, 1945.
- Graven S. N., Ormrod D. & Holcomb T. M. The effect of activity on concentration of blood lactate in newborn infants. *Pediatrics* 37: 278, 1966.
- Hasselbalch K. A. Respiratory foreign pressure in the newborn. *Bibl. Laryng.* 5: 219, 1904.
- Hey E. N. & Mount L. E. Heat losses from babies in incubators. *Arch. Dis. Childh.* 42: 75, 1967.
- Hill J. R. & Rahemtulla K. A. Heat balance and the metabolism rate of the newborn babies in relation to environmental temperature and the effect of age and of weight on basal metabolism rate. *J. Physiol.* 180: 239, 1965.
- Hill J. R. & Robinson D. C. Oxygen consumption in normally grown small-for-dates and large-for-dates newborn infants. *J. Physiol.* 199: 685, 1968.
- Jonas J. H. P., Van der Vliet J. J., De Groot C. J., Boersma E. R. & Meijers E. D. K. The metabolic rate in premature dysmature and sick infants in relation to environmental temperature. Nutrition Symposium. H. E. Stentfort Kroese, Leiden, Holland, 1968, pp. 201, 209.
- Karlberg P. Determinations of standard energy metabolism (basal metabolism) in normal infants. *Acta Paediat. Scand.* 41: Suppl. 89, 1952.
- Karlberg P., Moore R. E. & Olver T. A. Jr. The thermogenic response of the newborn infant to noradrenaline. *Acta Paediat. Scand.* 51: 84, 1962.
- Thermogenic and cardiovascular responses of the newborn baby to noradrenaline. *Acta Paediat. Scand.* 54: 225, 1965.
- Keele D. K. & Kay J. L. Plasma free fatty acid and blood sweat levels in newborn infants and their mothers. *Pediatrics* 37: 597, 1966.
- Levason H. & Swyer P. R. Oxygen consumption and the thermal environment in newly born as fat. *Biol. Neonat.* 7: 305, 1964.
- McCance R. A. & Strangeways W. M. B. Protein catabolism and oxygen consumption during starvation in infants, young adults and old men. *Brit. J. Nutr.* 8: 21, 1954.
- Melicher V., Drahota Z. & Hahn P. Changes in the blood levels of acetonecarboxylate and ketone bodies in newborn infants. *Biol. Neonat.* 9: 243, 1965.
- Ketone bodies in the blood of full-term newborn premature and dysmature infants and in infants of diabetic mothers. *Biol. Neonat.* 11: 23, 1967.
- Melicher V. & Wolf H. Postnatal changes in the blood serum content of glycerol and free fatty acids in premature infants. Influence of hypothermia and of respiratory distress. *Biol. Neonat.* 11: 50, 1967.
- Meysen J., Fekete M., Bata G. & Jarm I. The basal metabolic rate of premature infants. *Biol. Neonat.* 7: 11, 1964.
- Mount L. E. Basis of heat regulation in homeotherms. *Brit. Med. Bull.* 22: 84, 1966.
- Murkin J. R., Conklin R. & Marsh E. Energy metabolism of normal newborn babies with

This trend has also been observed here, however the RQ does not fall so deeply and there after rises earlier than previously reported. These differences call for two comments. The lowest RQ values previously published could be due to an environmental temperature lower than the neutral range. The earlier rising of the RQ values is likely related to the earlier feeding of the newborn in former investigations, the RQ values rise after the second day and the increase coincides with the milk feeding. In the present study milk feeding was started at the twelfth hour of age.

Considering the RQ differences between small for date and normal infants and despite a wide scattering of the values in both groups, the RQ is lower during the first 5 days in the small for date infants. These results could be usefully considered in relation to recent reports on lower blood glucose and carbohydrate storage in foetal malnutrition (3, 6, 9, 39, 40, 43). Moreover FFA, glycerol and ketone bodies in the blood rise rapidly to higher values in the small for date infants than in normal full term infants (27) hence lipids are mobilized and utilized to a greater extent in the former group. Oxygen consumption values in these two small groups are within the range of wider published investigations (1, 17, 24, 34). The mean value of  $\dot{V}O_2$  uptake in small for date infants is lower than in full term babies. According to the report of Sinclair et al (41) a higher value of oxygen consumption would be expected in small for-date infants: their extracellular fluids being probably diminished. On the contrary our results of oxygen consumption in small for-date infants are similar to the values in premature babies (29, 38). There is also the possibility of oxygen consumption decreasing as a starvation reaction from foetal malnutrition. In the small for date group oxygen consumption rises with age ( $r=0.58$ ) ( $p<0.001$ ). Contrarily oxygen consumption does not change significantly ( $r=0.18$ ,  $p=0.28$ ) in normal fullterm infants. During the writing of this paper Jonxis et al published extensive results of  $\dot{V}O_2$  uptake and RQ values in normal full term, premature and

small for date infants in relation to environmental temperature (19). In all three groups the RQ dropped during the first day of life and rose again from the third day onwards. On the third day of life the RQ was respectively 0.80 in full term, 0.78 in premature and 0.75 in small for date infants. Especially in the small for-date group, values close to 0.70 have been observed. In all groups, the RQ rises when larger quantities of food are given but feeding was started only on the second day of life. In many small for date babies there is a marked drop in the RQ combined with an increased  $\dot{V}O_2$  consumption during exposure to cold. The  $\dot{V}O_2$  uptake of the small for-date babies was initially 4.7 ml/kg/min and rose to 6.8 ml during the first 10 days. This agreement between the results of two independent studies which could be expected theoretically is encouraging when actually observed.

However the results obtained in these studies do not correspond with the oxygen consumption values in normally grown small for-date and large for date newborn infants in the recent study of June Hill & Robinson (18). They found no significant differences between these groups in agreement with the study of Sinclair et al (41). The differences might be of methodological origin. Since Hill & Robinson measured the oxygen consumption over a period of approximately 10 min at a number of different environmental temperatures and also in cold conditions during an interval between 3 hourly feeds but we determined our results after half an hour of sleep the steady state might have been more pronounced in the present study. The heat loss might also have been kept at a lower level. According to Jonxis et al the small for date infants show a more pronounced metabolic response to a cold environment.

## SUMMARY

Respiratory quotient and gaseous metabolism were measured in normal full term and small for-date infants during the first week of life. In both groups RQ dropped during the first day

## THE EFFECT OF DIURETICS IN LATE PREGNANCY ON THE NEWBORN INFANT

JØRGEN BENT ANDERSEN

*From the Department of Obstetrics A and Gynecology I (Heads D Trolle and B Sprøn-  
sen) and the Paediatric Department (Heads P Ploun J C Melchior and B Fris Hansen)  
Rigshospitalet Copenhagen Denmark*

The mechanism for the distribution of water between the mother and the foetus is not precisely known. Several investigators have found that administration of hypo- and hypertonic solutions intravenously to the pregnant mother has a prompt effect on the total osmotic pressure and the concentration of electrolytes in the foetus. Changes in the extracellular compartments of the mother are also accompanied by rapid changes in the fetal extracellular space (1, 12).

From a theoretical point of view it could be expected that administration of diuretics during the last part of pregnancy might have some effect on the water balance of the newborn infant indirectly by removing water and electrolytes from the mother or directly have an effect in cases where the diuretics freely pass the placenta barrier as e.g. the thiazid group has been shown to do (4).

Since the introduction in 1957 of the thiazides they have been widely used in the prophylaxis and treatment of toxemia of pregnancy and in the treatment of oedema in pregnancy (11, 13, 15). In the available literature however there has been no reports of disturbances in the body water compartments or in the mineral metabolism of the newborn infant.

The aim of the present study was to in-

vestigate if the administration of the two diuretics of the thiazid group Bendroflumethazid and Chlortalidon during the last part of pregnancy until delivery has any detectable effect on the water and mineral metabolism in the newborn infant.

### MATERIAL

This comprised 16 infants: a control group of 8 full term infants born of normal mothers and a group of 8 full term infants born of mothers who had been treated with Bendroflumethazid or Chlortalidon during a various length of time until delivery. In all cases the diuretic was given until the day of delivery. In the treated group there was an additional administration of potassium salt. The indication for the diuretical treatment was abnormal weight gain and oedema; in no cases toxemia was present. The type of drug, the dose and the duration of administration is shown in Table 1. There was no sign of dehydration.

Within a few hours before or after delivery blood samples were drawn from the mother for determination of sodium, potassium and chloride concentration as well as total osmolality. Preliminary observations have shown that these values vary very little during the hours before and after delivery. In the newborn infants the total body water (TBW), the extracellular water (ECW), the concentrations of sodium, potassium and chloride and the osmolality was determined 2-4 hours after birth. (In one case 1620/69 the determination was made 9 hours after birth.)

The values in the 2 groups were compared.

### METHODS

The total body water was determined by the dilution method using deuterium oxide (D.O. 99.78 g/100 g

This work has been supported by grants from the Danish Medical Research Council and the D. Marshall Foundation.

- special reference to the influence of food and of crying *Amer J Dis Child* 29 1 1925
- 32 Novák M, Melichar V & Hahn P Postnatal changes in the blood serum content of glycerol and fatty acids in human infants *Biol Neonat* 7 179 1964
  - 33 — Changes in the content and release of glycerol in subcutaneous adipose tissues of newborn in fants *Biol Neonat* 8 253 1965
  - 34 Oliver T K Jr & Luryberg P Gaseous metabolism in newly born human infants. The effects of environmental temperature and 15 oxygen in the inspired air *Amer J Dis Child* 105 427, 1963
  - 35 Parmelee A H, Bruck K & Bruck M Activity and inactivity cycles during the sleep of premature infants exposed to neutral temperature *Biol Neonat* 4 317 1962
  - 36 Pernow B & Gents J Free fatty acids and glycerol in plasma of newborn infants *Acta Paediat Scand* 54 198 1965
  - 37 Scopes J W Metabolic rate and temperature control in the human baby *Brit Med Bull* 22 88 1966
  - 38 Scopes J W & Ahmed I I Minimal rates of oxygen consumption in sick and premature newborn infants *Arch Dis Childh* 41 407 1966
  - 39 Shelley H J Glycogen reserves and their changes at birth and in anoxia *Brit Med Bull* 17 137 1961
  - 40 Shelley H J & Neligan G A Neonatal hypoglycaemia *Brit Med Bull* 22 34 1966
  - 41 Sinclair J C, Scopes J W & Silverman W A Metabolic reference standards for neonate *Pediatrics* 39 724 1967
  - 42 Silverman W A & Agate F J Variation in cold resistance among small newborn infants *Biol Neonat* 6 113 1964
  - 43 Stern L, Sourkes T L & Rauh N The role of the adrenal medulla in the hypoglycaemia of foetal malnutrition *Biol Neonat* 41 129 1967

Submitted Febr 8 1969

Accepted April 6 1970

(P K.) Dept of Paediatrics  
Göteborgs Barnsjukhus  
413 46 Göteborg  
Sweden

**Key words** Respiratory quotient metabolic rate full term newborn infants small for date newborn infants

Table 2b The values of body compartments serum electrolytes and osmolality in infants and in mothers who has been treated with diuretics in late pregnancy

Case no	Sex	Weight	TBW (%)	ECW (%)	Sodium mEq/l		Potassium		Chloride		Osmolality	
					Inf	Mother	Inf	Mother	Inf	Mother	Inf	Mother
A 1425/69	♂	3 680	76.3	37.4	139	138	4.5	3.9	111	109	287	280
A 1432/69	♀	3 660	73.8	44.5	142	140	4.2	3.6	108	106	280	284
A 1531/69	♂	2 740	75.3	—	138	136	3.9	3.4	107	105	285	271
A 1603/69	♀	3 090	74.2	26.8	145	148	3.7	3.4	111	111	296	307
A 1580/69	♀	3 410	70.8	40.4	140	144	4.5	3.6	101	101	282	282
A 1111/69	♂	3 600	74.0	38.1	140	140	4.4	3.7	105	102	278	285
A 1560/69	♂	4 560	69.7	34.9	139	139	4.8	3.8	103	104	280	287
A 1620/69	♀	3 410	76.7	34.3	144	141	3.9	3.7	103	102	290	290

The osmolality in the mothers and the newborn infants in the untreated control group is shown in Table 2a and the values for the group treated with diuretics is shown in Table 2b. The mean values for the two groups of infants is shown in Table 3 and the mean values for the two groups of mothers in Table 4.

The TBW of the infants in both groups was nearly identical. The ECW in the treated group was slightly reduced as well as the osmotic and the sodium, potassium and chloride concentrations. This reduction was not statistically significant using the Student's *t* test.

Among the mothers there was a trend towards higher values of sodium concentration and of osmolality and towards lower values for potassium and chloride in the treated group compared to the control group. The transplacental difference in sodium concentrations was larger in the control group (3.8 mEq/l) than in the treatment group (0.1 mEq). This difference is significant with a *p* value less than 0.001 using the Student's *t* test.

The changes observed in the infants of the treated group were not correlated to the duration of administration of diuretics and no comparison between the two diuretics was possible.

## DISCUSSION

The values for TBW and ECW in the control infants correspond well to those found by others (4, 6, 100, 101, 102, 103). The values for the sodium

and potassium concentrations as well as for the osmolality in the infants compared to the mothers is similar to the findings of other investigators (7, 10, 16).

The dominant action of the thiazides is to increase the renal excretion of sodium and chloride and an accompanying volume of water mostly derived from the extracellular compartment. This is the result from inhibition of the

Table 3 The mean values for body compartments serum electrolytes and osmolality in newborn infants from mothers with and without diuretical treatment in late pregnancy

Mean values	Without diuretics	With diuretics
Weight, g	3 583	3 513
TBW	74.0	73.9
ECW	40.3	38.1
Sodium mEq/l	142.6	140.3
Potassium mEq/l	4.61	4.24
Chloride mEq/l	107.8	106.1
Osmolality mosmol/l	282.6	284.8

Table 4 The mean values of serum electrolytes and osmolality in mothers with and without diuretical treatment in late pregnancy

Mean values	Without diuretics	With diuretics
Sodium mEq/l	148.8	140.8
Potassium mEq/l	3.86	3.64
Chloride mEq/l	107	105
Osmolality mosmol/l	282.5	285.8

Table 1 Dose of diuretics and the number of days before delivery it was administered

Chlortalidone Hygroton (D) 100 mg  
Bendroflumethazid  
Centyl (D) 2.5 mg c.h.c.L 573 mg

## Case no

- A 1435/69 Chlortalidone 100 mg in 36 days followed by  
Bendroflumethazid in 48 days  
A 1452/69 Bendroflumethazid 10 mg in 10 days  
A 1531/69 Bendroflumethazid 2.5 mg in 20 days  
A 1603/69 Bendroflumethazid 5 mg in 28 days  
A 1680/69 Bendroflumethazid 2.5 mg in 38 days  
A 1111/69 Bendroflumethazid 2.5 mg in 24 days  
A 1560/69 Bendroflumethazid 5 mg in 6 days  
A 1620/69 Chlortalidone 100 mg in 18 days

supplied in ampoules of 25 g which was transferred to 5 ml ampoules after addition of 9 mg NaCl/ml and sterilized. Approximately 15 ml D<sub>2</sub>O per kg body weight were given through an umbilical vein catheter with calibrated syringes. Equilibration was reached at 2 hours and 2 plasma samples for double determinations were drawn through the catheter at 2 / and 3 hours after injection and stored at -10 °C in sealed tubes each containing 3/4 1 ml plasma. Vacuum sublimation of the plasma samples were performed (9) and the concentration of D<sub>2</sub>O in H<sub>2</sub>O was measured in an infrared spectrophotometer (Perkin Elmer 421) by using the absorbance at 3.98  $\mu$  (2513 cm<sup>-1</sup>) (14, 17).

The concentration of D<sub>2</sub>O in H<sub>2</sub>O was in the range of 0.1-0.2 vol % and as standard was used D<sub>2</sub>O in distilled water in the concentrations of 0.1, 0.2, 0.3 and 0.4 vol %.

The procedure for measuring an unknown sample was as follows: the 2 standards on either side of the expected value was determined and the concentration of the unknown sample was interpolated.

The 2 standards always preceded the sample. If more than 3 unknown samples were analyzed a new set of standards was read at the conclusion of the analysis and for the interpolation the average of standards before and after analysis was used.

TBW is calculated from the equation

$T = \frac{\text{injected volume of D}_2\text{O}}{\text{conc. of D}_2\text{O in plasma}}$

No correction was made for the impurity of D<sub>2</sub>O, the exchange of labile hydrogen atoms of the solid constituent of the body (9) or for the excretion of D<sub>2</sub>O before equilibrium.

The extracellular water was determined by using the thiosulfate volume of dilution in a modification (3) of the micro-method originally described by Newman et al. (6). A 5% solution of sodium thiosulfate was used and 1.5 ml/kg body weight were injected through the umbilical vein catheter just before the injection of D<sub>2</sub>O. Blood samples for analysis were drawn from the heel and the values 20, 30, 40, 50 and 60 min after injection were determined. From these the zero value was calculated using the least square method. The ECW was expressed in per cent of total body weight just prior to injection in the same manner as with TBW.

The osmolality was determined by an L-shaped osmometer and the coefficient of variation on a double reading was 0.33%.

## RESULTS

The effect of diuretics on the pregnant mother was in all cases shown by a weight loss. A side effect of thiazid is a reduced excretion of uric acid and an elevation of the serum uric acid. In a small trial the mean value of serum uric acid was 7.2 mg/100 ml in the treated group against 4.2 mg/100 ml in 6 mothers in late pregnancy who had received no treatment with diuretics.

The values for body water compartments in the newborn infants and the serum concentrations of sodium, potassium and chloride and

The osmolality was measured at the Medical Physiologic Institute C, Rådmandsgade 71, Copenhagen N. Head Professor N. A. Thorn.

Table 2a The values of body compartments, serum electrolytes and osmolality in the control group

Case no	Sex	Weight	TBW ( )	ECW ( )	Sodium mEq/l		Potassium		Chloride		Osmolality	
					Infant	Mother	Infant	Mother	Infant	Mother	Infant	Mother
A 1466/69	♂	2.825	79.9	39.3	151	140	4.5	3.8	114	108	324	281
A 1470/69	♂	2.560	79.0	46.1	144	137	4.9	4.0	106	103	285	277
A 1489/69	♂	4.880	67.4	34.8	139	136	4.6	3.8	106	106	277	272
A 1474/69	♂	3.715	72.7	38.0	140	137	4.8	4.2	107	107	290	291
A 1481/69	♀	3.090	81.0	41.7	141	136	4.0	3.3	104	106	290	289
A 1516/69	♂	3.740	73.2	44.0	143	143	4.8	3.9	108	107	282	285
A 1549/69	♂	3.860	70.6	44.9	140	137	4.7	3.9	106	105	275	275
A 1592/69	♀	4.000	68.2	33.7	143	144	4.6	4.0	111	112	286	290

- 16 Thomsen B. Electrolyte and acid base balance in fetal and maternal blood. *Acta Obstet Gynec Scand* 45 Suppl 8
- 17 Turner M D, Neely W A & Hardy J D. Rapid determination of deuterium oxide in biological fluids. *Appl Physiol* 15 309 1960

Neonatal Department  
Rigshospitalet  
2100 Copenhagen Ø  
Denmark

*Key words:* Body fluids, diuretics, newborn infant, pregnancy

Submitted Febr. 2 1970

Accepted April 20 1970



tubular mechanism of electrolyte reabsorption and a well known side effect is hypocalcemia

In the mothers of the treated group there was a clinical effect with loss of weight and disappearance of edema. From the doses used an effect on the electrolyte concentrations in the mothers blood would not be expected, actually in this study insignificantly higher values for sodium and osmolality were found.

In infants born to mothers treated with diuretics some changes in water and mineral metabolism were observed as compared to the control group. These observations are not statistically significant, there is however a general trend in all 6 parameters pointing in the same direction. TBW was only slightly reduced, ECW was somewhat more reduced as well as the concentrations of sodium, potassium and chloride and the osmolality. These findings could be interpreted as the result of a slight water and salt depletion. The statistically confirmed observation that the concentration gradient from infants to mother for sodium and the difference of the osmolality is lessened in the diuretic group is in agreement with this interpretation.

The question, if there is 1) an indirect effect via changes in the mother in the water and mineral metabolism caused by the diuretics or 2) a direct action on the placenta or 3) a direct renal effect on the foetus, cannot be precisely answered. But the fact, that thiazides are able to pass the placenta barrier and that the changes between the two groups of infants are more marked than the changes between the mother groups could either mean that there is an effect on the kidney of the foetus, a direct effect on the placenta, or a combination of the two effects.

## SUMMARY

The changes in water and mineral metabolism in newborn infants born of mothers, who have been treated in late pregnancy with diuretics, was investigated. It was found that all 6 para-

meters the total body water, the extracellular water, the serum concentrations of sodium, potassium and chloride and the osmolality all pointed in the direction that there was a slight salt and water depletion in the infants from the mothers, treated with diuretics.

The biochemical observations were not clinically detectable.

## REFERENCES

- 1 Battaglia F, Prytowsky H, Smisson C, Hellegers A & Bruns P. The effect of the administration of fluids intravenously to mothers upon the concentration of water and electrolytes in plasma of human fetuses. *Pediatrics* 23:2 1960.
- 2 Friis Hansen B. The extracellular fluid volume in infants and children. *Acta Paediatr Scand* 43:444 1954.
- 3 — Body water compartments in children. *Pediatrics* 28:169 1961.
- 4 Garnet J D. Placental transfer of chlorothalidate. *Obstet Gynec* 23:123 1963.
- 5 Glädde, H. Die Bestimmung des extracellulären Flüssigkeitsraumes bei Kindern mit Hilfe von Thiosulfat. *Z Ges Exp Med* 141:301 1966.
- 6 Newman E W, Gilman A & Phillips F S. Renal clearance of thiosulfate in man. *Bull Johns Hopk Hosp* 79:229 1946.
- 7 Mellor D J, Cochran F & Lees M M. Distribution of ions and electrical potential differences between mother and fetus in the human at term. *J Obstet Gynec* 76:993 1969.
- 8 Oiler M. Body water of newborn infants of diabetic mothers. *Acta Endocr* 34:261 1960.
- 9 Schloerb P R, Friis Hansen B, Edelman I S, Solomon A K & Moore F D. The measurement of total body water in the human subject by deuterium oxide dilution. *J Clin Invest* 23:1246 1950.
- 10 Seeds E. Water metabolism of the fetus. *Amer J Obstet Gynec* 92:727 1965.
- 11 Sele V. Benzylhydroflumethazid (Cenyl Leo) virkning på svangerskabsødemer. *Ugeskr Læ* 122:1017 1960.
- 12 Sellar M J. The effects of maternal overhydration on the rat foetus. *Quart J Exp Physiol* 48:292 1963.
- 13 Shuback F & Tatum H. Prophylactic use of Chlorothalidone in pregnancy. *Obstet Gynec* 23:353 1964.
- 14 Stansell M & Mopica L. Determination of body water content using trace level of deuterium oxide and infrared spectrophotometry. *Chm Pharm* 14:1112 1968.
- 15 Tatum H J & Waterman M A. The prophylactic and therapeutic use of thiazides in pregnancy. *Proctonomer* 24:101 1961.

- 16 Thakur B. Electrolyte and acid base balance in fetal and maternal blood *Acta Obstet Gynec Scand* 45 Suppl 8
- 17 Turner M D, Neely W A & Hardy J D. Rapid determination of deuterium oxide in biological fluids *Appl Physiol* 15 309 1960

Neonatal Department  
Rigshospitalet  
2100 Copenhagen Ø  
Denmark

*Key words* Body fluids, diuretics, newborn infant, pregnancy

Submitted Febr. 2 1970

Accepted April 10 1970

## THREE CASES OF DISSEMINATED INTRAVASCULAR COAGULATION

GUNILLA BERGLUND

*From the Department of Paediatrics University of Göteborg Göteborg Sweden*

Disseminated intravascular coagulation (DIC) should be considered in relation to sudden deterioration in the course of various diseases in childhood as well as with unexplained bleeding conditions. If DIC can be diagnosed and interrupted before extensive thrombosis and tissue damages have occurred a prompt recovery can be obtained.

The mechanism in DIC is an extensive formation of fibrin in the vascular system especially in the small vessels. During the process, coagulation factors are consumed, above all fibrinogen, platelets factor V and factor VIII. There is an increased activation of plasminogen to plasmin which lyses part of the formed fibrin and fibrinolytic split products are released. In the presence of these and thrombocytopenia continued fibrin formation will result in abnormal clotting which has been observed to damage the erythrocytes and thus give rise to hemolytic anemia (6-24).

The sudden loss of coagulability of the blood was first observed and described in obstetrical practice (9). Further investigations demonstrated that in this situation thromboplastic material had entered the maternal circulation (23). The material which initiates the DIC is often amniotic fluid with debris, but tissue fragments and bacterial products can also be responsible (22).

The occurrence of DIC concomitant to various conditions has been much discussed for example in conjunction with hemolytic uremic

syndrome, hemolytic anemia, malignant diseases, sepsis, burns, snake bites and many other conditions (7, 8, 12, 13, 16). However, there is a striking resemblance between the suddenly developing changes including clotting defects found in some diseases and those changes obtained in experimental animals by injecting thromboplastic material (11). There can be a rapid change in the general condition often accompanied by shock. Definitive diagnosis of DIC can occasionally be made at biopsy, otherwise only in patients who die in an acute phase of the disease, because the thrombi in the small vessels only exist for a short time.

Three cases treated at Children's Hospital, Göteborg are here presented. The clinical findings indicated DIC and quantitative normalization of the clotting factors was obtained as soon as the formation of excessive fibrin was interrupted.

### CASE REPORTS

#### *Case 1*

59 04 30 Y S A previously healthy 4 year-old girl was admitted to Children's Hospital, Göteborg because of abdominal pain and vomiting for 4 days. The abdominal pain had started suddenly and was of intermittent type increasing in severity. On admission she was pale, tired and complaining of diffuse pain in the abdomen. She ran a slight fever. Around the neck she had a few petechiae. In the left upper quadrant of the abdomen a solid rounded tumor was palpated extending 5 cm below the arch. Urography demonstrated that the left kidney was pushed down by a tumor but the appearance of the kidney was not otherwise influenced. The laboratory findings

Table 1 Laboratory findings in three patients with disseminated intravascular coagulation

Case 1: 1 Day of admission 2 3 days after operation Case 2: 1 At 12 hours of age 2 At 3 days of age  
Case 3: 1 Day before the renal biopsy 2 Day of deterioration (3 days after biopsy) 3 3 days later

Investigation	Case 1		Case 2		Case 3		
	1	2	1	2	1	2	3
Fibrinogen mg/100 ml	0	530	110	260	920	90	530
Number of platelets/mm <sup>3</sup>	20 000	388 000	80 000	180 000	325 000	60 000	190 000
P & P	26	56	—	—	—	—	—
Prothrombin	—	—	27	43	67	48	79
Factor V	33	80	58	71	96	42	89
Factor VIII %	21	92	54	78	86	28	67
Fibrinolytic activity	—	—	—	—	—	—	—
Fibr split products	+	—	(+)	—	—	+	—

Blood collected with Eponox aminocaproic acid

(Table 1) demonstrated a moderate anemia. No fibrinogen could be demonstrated by electrophoresis or clotting methods (3) but with immunodiffusion analysis a faint fibrinogen precipitate was observed. A moderate decrease of PF values (19) factor V (20) and factor VIII (4) was observed but factor IX (4) was unchanged. The number of platelets was diminished. No fibrinolysis could be demonstrated (1) but with immunodiffusion methods (2) fibrinolytic split products were identified in serum. The test of liver function was normal. In the urine some proteinuria was noted occasionally but the sediment as well as the osmolality was normal. X ray of the skeleton and the lungs did not show metastases.

During the days following admission the condition of the girl was unchanged but she developed a moderately increased bleeding tendency with nose bleedings and a few subcutaneous haematomas. As the clotting disturbances could have been induced by the tumor it was decided to remove it.

Early in the morning of the operation day she obtained 400 ml platelet rich plasma (each ml contained platelets from 10 ml normal blood). 5 hours later she received preoperatively 2 g fibrinogen (Habi Stockholm) and 400 ml fresh blood. No excessive bleeding occurred during the operation (3 Hagberg) but she was given another 400 ml fresh blood 1 g fibrinogen and 200 ml platelet rich plasma and 2 hours after the operation 1 g epsilon aminocaproic acid.

A retroperitoneal capsulated rounded tumor with a diameter of about 12 cm was extirpated after difficulty in freeing it from the surrounding tissues especially from the aorta and its branches. The tumor had developed from the left suprarenal gland which was also extirpated. The weight of the tumor was almost 900 g. The sectioned surface was dark red and concave but no solid clots were visible. At microscopic examination it was noted that the tumor consisted of bundles of tumor cells arranged in an alveolar structure filled with bleedings and vessels. The pathological diagnosis was neuroblastoma of the

type called sympathoganglioneuroma tenui fibrillare (J. Melligren).

In order to determine its clotting action a saline extract of the tumor was made after removing the blood by repeated washing. For comparative purposes similar extracts were made from normal suprarenal glands or brain tissue and tissue from neuroblastomas from other patients. The fibrinolytic activity of all of these tumor-extracts was almost none when tested with the fibrin plate method (1). The thromboplastic activity of the different extracts was measured by noting their effect on normal plasma in the recalcification test. The tumor tissue from the patient showed as potent thromboplastic activity as the brain tissue while the tumor extracts from the other patients as well as the normal suprarenal tissue hardly influenced the recalcification time.

Postoperatively the patient did well and the clotting factors reached normal levels. Fibrinogen and factor VIII increased to about twice normal values during the two subsequent days thereafter they normalized. Because of the malignancy of the tumor the patient received postoperative radiation treatment as well as cytostatic drugs for a period of 14 days. Two years later she was in good health and showed no signs of relapse as regards to the tumor or the clotting defects.

#### Case 2

69.07.22. Girl R. This newborn baby was sent to Children's Hospital directly after delivery because of extensive bleeding from an accidental cut on her face. She was the first child of a healthy mother. During the normal delivery the foetus developed intracranial asphyxia and an emergency cesarean section was performed. During this procedure a cut in the left parietal region of the infant was accidentally made at the incision of the uterus. The wound was 5 cm long and extended down to the bone. It was found necessary to suture the wound. During the following hours a large hematoma developed in infiltrating the soft tissue of the neck. The general

condition of the infant deteriorated she went into respiratory distress and intubation was performed. During the next 12 hours the bleeding persisted. The infant obtained 600 ml fresh blood to compensate for the blood loss. The wound was opened in search of a bleeding vessel. None was found but blood was oozing from everywhere. A blood sample drawn at that time demonstrated a moderate decrease of prothrombin (16) factor V and factor VIII and decrease in number of platelets (Table 1). The diagnosis DIC was possible and the child was started on heparin treatment (200 U/kg body weight every 6 hours). Four hours later she was given epsilon aminocaproic acid (0.1 g/kg body weight). The bleeding ceased shortly after the administration of heparin and there was no need for further blood transfusion. Thereafter the child recovered quickly and the heparin treatment was discontinued after 2 days. 12 hours later the levels of the clotting factors were normal. She was taken out of the respirator, the tube was removed and 2 weeks later she was completely well.

### Case 3

560129 G A This girl 12 years of age had previously been healthy except for a urinary tract infection at the age of 8 in 1964 probably cystitis. Four months prior to admission she was ill with moderate fever, back pain and bacteriuria which persisted for almost 2 months. She had courses of treatment with sulpha, tetracycline and nitrofurantoin. Urography at that time was normal. Two months later she developed signs of renal insufficiency with proteinuria, oedema, increased non protein nitrogen and hypertension. She was admitted to Children's Hospital, Göteborg. For evaluation of her disease an open renal biopsy was essential. Except for a high fibrinogen content the investigation of clotting factors the day previous to the kidney biopsy was completely normal. Biopsy was performed without complication. The histological diagnosis was sub-acute glomerulonephritis with endothelial and epithelial proliferation in the capillary loops of the glomeruli. In the tubuli degenerative changes were demonstrable. Three days after the operation the condition of the patient suddenly deteriorated and she went into pre-shock. She had contracted a urinary tract infection with *E. coli* and enterococci, hemolytic anemia and anuria. A slight increase in the bleeding tendency was observed. She had an oozing bleeding from the operation wound and from punctures in her fingers after blood tests. The coagulation investigation demonstrated a decrease of the previously very high fibrinogen content, factor V and factor VIII as well as the number of platelets. No fibrinolytic activity was noted but fibrinolytic split products were observed with immunodiffusion methods (Table 1). She was given plasma transfusion (20 ml/kg body weight), antibiotics and then started on a heparin treatment (125 U/kg body weight every 6 hours). Three days later normalization of the clotting factors had occurred, the hemolysis had disappeared and the amount of urine was increasing. The heparin treatment was continued for about 2 months

with a short interval when she needed a surgical cleaning of the biopsy wound which demonstrated very poor healing with various bacterial infections. After the surgical cleaning it healed well. Her primary disease was treated with cortisone and 6-mercaptopurine. During the following months she improved though she continuously demonstrated signs of serious kidney damage. Later she went into renal failure and died 6 months afterwards. Examination of the kidneys demonstrated the same changes as earlier found in biopsy.

## DISCUSSION

Of these 3 patients the bleeding tendency dominated the symptoms only in case 2. Deterioration of the general condition was the main symptom in the others possibly due to the blockage of the microcirculation. The deterioration was slowly progressive in case 1 and rapid in case 3. These 2 patients also had a slightly increased bleeding tendency which could easily have been overlooked. In case 1 the change consisted of a few petechiae and in case 3 oozing from the operation wound and finger punctures after blood tests. In all 3 cases the coagulation pattern showed reduction of prothrombin, factor V and factor VIII as well as number of platelets. These changes were most obvious in case 1. In case 3 the levels were not as strikingly low as in case 1, however in relation to the prebiopsy figures a considerable decrease was apparent. The very high prebiopsy fibrinogen content can be explained by the renal disease. In case 2 the absolute figures are obscured by the large amount of transfused blood.

For practical purposes the diagnosis DIC can be strongly suspected by a low platelet count combined with a decreased level of fibrinogen when one or the other of these findings cannot be related to the basic condition. The demonstration of the changes in the other coagulation factors is time-consuming and often requires specialists.

Of the differential diagnosis acute thrombocytopenia and fibrinolysis should be considered. In acute thrombocytopenia the fibrinogen level is normal and the platelet count is usually

lower than that in DIC. Values below 20 000/ $\mu\text{m}^3$  are common in thrombocytopenia whereas in DIC the decrease is usually not as great. In patients with primary fibrinolysis the bleeding tendency always dominates the symptoms. Moderate increased fibrinolytic activity can sometimes be demonstrated in DIC in primary fibrinolysis the fibrinolytic activity is pronounced and followed by a decrease of the level of fibrinogen and also of other clotting factors. The number of platelets is however always unaffected.

The initiating cause of the intravascular clotting in these 3 patients varied. In case 1 the tumor demonstrated a very high thromboplastic activity. It is known that malignant tumors can promote intravascular coagulation but there has been much speculation about the exact mechanism. Blix & Jacobsen (5) suggested that thromboplastic material from a tumor was released into the blood stream. In case 1 fragments of the tumor in the blood stream would very probably give rise to a severe intravascular clotting because of the potent thromboplastic activity of this tumor. The contrary action of other neuroblastoma tissue is puzzling.

Case 2 had bleeding tendency immediately after the delivery. The child received the laceration some minutes earlier when it was still *in utero* surrounded with meconium-containing amniotic fluid. A speculation is that some of the amniotic fluid was sucked into the opened vessels of the foetus. The thromboplastic effect of meconium-containing amniotic fluid is known (22). A contributing factor could be that the infant had intrauterine asphyxia.

In case 3 the renal biopsy did not seem to be the cause of DIC. The urinary tract infection with released endotoxins from *E. coli* could be the trigger mechanism of DIC. The conditions were perhaps particularly favourable for the accessibility of the toxin in the newly biopsied kidney. It might have started as a Schwartzman reaction which is known to be mediated by intravascular clotting (15). Hardaway (11) also states that high levels of blood fibrinogen in pregnancy promote the Schwartz-

man reaction and in case 3 it was demonstrated that her fibrinogen level was very high.

The recovery of the 3 patients was prompt after interrupting the intravascular coagulation in case 1 through extirpation of the tumor in cases 2 and 3 through immediate treatment with heparin. In case 3 treatment was continued for almost 2 months with the hope of preventing further local fibrin deposits in the kidney as a result of an antigen antibody reaction (14) in the kidney already severely damaged by the subacute glomerulonephritis. However after mutual improvement the disease ran its progressive course.

The treatment of DIC should be directed towards preventing further coagulation by giving heparin in ordinary doses. Heparin must be given in spite of the bleeding tendency of the patient. In case 1 the DIC was interrupted by extirpation of the tumor after massive replacement therapy but it might have been safer to give her preoperative heparin treatment. However failure with heparin treatment in DIC has also been reported (10) and the use of lytic substances such as urokinase has been suggested (13). The administration of lytic substances should be accompanied by continuous clotting studies of the patient.

## SUMMARY

Disseminated intravascular coagulation (DIC) was a complication in 3 patients with different primary diseases. The probable initiation of the DIC was a thromboplastic active tumor, a laceration wound at caesarean section with amniotic embolism in the foetus and probable endotoxin release in a newly kidney biopsied patient with subacute glomerulonephritis. The DIC was interrupted in the first patient by extirpation of the tumor, in the other two by treatment with heparin in spite of bleeding tendency.

The diagnosis can be strongly suspected by demonstrating a decrease of the fibrinogen content combined with a diminished number of platelets. A normalization of the condition can

occur after interruption of the intravascular clotting by treatment with heparin

## REFERENCES

- 1 Astrup T & Mullertz E The fibrin plate method for estimating fibrinolytic activity *Arch Biochem* 40 346 1952
- 2 Berglund G Immunoelectrophoretic studies of fibrinogen plasmin and lytic products of fibrinogen and fibrin *Int Arch Allergy* 21 193 1962
- 3 Bergstrom K, Blomback B & Kleen G Studies on the plasma fibrinolytic activity *Acta Med Scand* 168 291 1960
- 4 Biggs R, Eveling J & Richards G The assay of antithrombotic globulin activity *Brit J Haemat* 1 20 1955
- 5 Blix S & Jacobsen C D The defibrination syndrome in a patient with haemangio-endothelial sarcoma *Acta Med Scand* 173 377 1963
- 6 Bull H S, Rubenberg M L, Dacre J V & Brain M C Microangiopathic haemolytic anaemia mechanism of red cell fragmentation in vitro studies *Brit J Haemat* 14 643 1963
- 7 Corrigan J J, Ray W L & May N Changes in the blood coagulation system associated with septicemia *New Eng J Med* 279 831 1963
- 8 Craig J M & Gitlin D The nature of the hyaline thrombi in thrombotic thrombocytopenic purpura *Amer J Path* 33 231 1957
- 9 Deele J B A case of fatal haemorrhagic diathesis with premature detachment of the placenta *Amer J Obstet* 44 785 1901
- 10 Doughten R M & Pearson H A Disseminated intravascular coagulation associated with Aspergillus endocarditis *J Pediatr* 73 576 1968
- 11 Hardaway R M *Syndromes of disseminated intravascular coagulation* Charles C Thomas Publ Springfield Ill USA 1966
- 12 Hathaway W E, Mull M M & Pechet G S Disseminated intravascular coagulation in the newborn *Pediatrics* 43 233 1969
- 13 Lanzkowsky Ph & McCrory W Disseminated intravascular coagulations as a possible factor in the pathogenesis of thrombotic microangiopathy (hemolytic uremic syndrome) *J Pediatr* 70 460 1967
- 14 McCluskey R T, Benicerraf T B, Potter J L & Miller F The pathologic effects of intravenously administered soluble antigen-antibody complexes I Passive serum sickness in mice *J Exp Med* 111 181 1960
- 15 McKay D O, Gitlin D & Craig J M Immunochemical demonstration of fibrin in the generalized Shwartzman reaction *Arch Path* 67 270 1959
- 16 McKay D O, Mansell H & Hertig A T Carcinoma of the body of the pancreas with fibrin thrombosis and fibrinogenopenia *Cancer* 6 682 1953
- 17 Menakey C Diagnosis and treatment of intra-vascular coagulation *Brit J Haemat* 15 523 1968
- 18 Noren J A specific two stage assay of prothrombin *Scand J Clin Lab Invest* To be published
- 19 Owren P A & Aas K The control of diathermol therapy and the quantitative determination of prothrombin and proconvertin *Scand J Clin Lab Invest* 1 201 1951
- 20 Quick A J & Stefanni M The concentration of the labile factor of the prothrombin complex in human dog and rabbit blood its significance in the determination of prothrombin activity *J Lab Clin Med* 33 819 1948
- 21 Reid H A, Chan K E & Thean P C Prolonged coagulation defect (defibrination syndrome) in Malayan viper bite *Lancet* 1 671 1963
- 22 Reid D E, Weiner A E & Roby C C Intra-vascular coagulation and afibrinogenemia the presumptive lethal factors in the syndrome of amniotic fluid embolism *Amer J Obstet Gynec* 66 465 1953
- 23 Verstraete M & Vermeylen J Acute and chronic defibrination in obstetrical practice *Thrombotic Diathesis Haemorrh* 20 444 1968
- 24 Leading article Microangiopathic haemolytic anaemia *Scott Med J* 14 187 1969

Submitted Aug 29 1969

Accepted April 2 1970

Dept of Paediatrics  
Goteborgs Barnaklinik  
413 46 Goteborg  
Sweden

**Key words** Blood coagulation disorders amniotic fluid embolism neuroblastoma Shwartzman phenomenon

## A PATIENT WITH HEREDITARY GALACTOKINASE DEFICIENCY

A DAHLQVIST I GAMSTORP and H MADSEN

*From the Chemical Research Department of the Hospital University of Lund Lund  
and Department of Paediatrics Central Hospital Jönköping Sweden*

The normal metabolism of galactose takes place through a transformation to glucose which occurs in 3 steps each catalyzed by a separate enzyme. The enzymes involved are

- 1 Galactokinase (ATP  $\rightarrow$  galactose 1 phosphate transferase E.C. 2.7.1.6)
- 2 Galactose 1 phosphate uridylyltransferase (UDPGlucose  $\rightarrow$  D galactose 1 phosphate uridylyltransferase III C.2.7.7.12) and
- 3 UDPGalactose 4 epimerase or galactowaldenase (UDPGlucose 4-epimerase E.C. 5.1.3.2)

No patient has yet been reported with deficiency of the third enzyme. Deficiency of the second enzyme causes the classical form of hereditary galactosemia (11-19, 20) clinically characterized by acute onset of jaundice and convulsions during the neonatal period later followed by evidence of liver cirrhosis, mental retardation and cataracts. In 1965 Gitzelman (13) reported the first case of hereditary galactokinase deficiency. This case occurred in an adult who already in infancy had been recognized as having galactose diabetes (10); the patient had cataracts but showed no signs of liver involvement or of mental retardation. The same metabolic defect has been demonstrated in one sister of this patient (14, 15), another sister and a brother have similar symptoms but have not been investigated biochemically. Hereditary galactokinase deficiency has also been reported in a newborn infant in an Austrian

family (31) unrelated to the previously described Swiss family; both families are gypsies. Mayes & Guthrie (22) during screening of 642 individuals from a mixed North American population found 6 persons in whom the galactokinase activity of the red blood cells was about half of the normal value; these individuals were judged as heterozygotes for galactokinase deficiency. To our knowledge no non Gypsy patients have however previously been reported with galactosemia due to galactokinase deficiency.

### CASE REPORT

The patient, a boy born on June 24, 1969, is the third child of healthy unrelated parents. His brother and sister are healthy. Pregnancy, labour and delivery were normal. Birthweight was 3760 g. The boy was breast fed for the first few days and then received a formula (Milkotal® 140 g 5 times a day) containing 9-10% lactose. He took 40-50 g eagerly but then appeared tired and the mother had some difficulties to make him take the whole meal each time. His weight gain was a little slow and at age 3 weeks his weight was 90 g below the birth weight. Other than this he was symptom free.

At 3 weeks of age he was admitted to the Department of Paediatrics Jönköping because a test for galactosemia (Guthrie's microbiological inhibition assay) performed in the neonatal period as a screening procedure was then reported abnormal. He was a healthy looking normally developed infant. Physical examination including a careful neurological examination revealed no abnormalities. Particularly no liver enlargement could be demonstrated at any of the examinations. The first ophthalmological examination which was performed when the boy was

The ophthalmological examinations were performed by Dr I. Anger.



Table 1 Galactokinase activity in hemolyzed red blood cells (radiochemical method (22-29) from the patient his relatives and a number of controls

The controls have been divided into two groups according to age: one infant group (2-4 months) with the higher galactokinase activity characteristic for the newborn period (25) and one group of adults and children (2-67 years). The values found in the controls agree well with earlier reports (22-25).

Subjects	Age	Galactokinase (units/ml erythrocytes)
Patient	3 mo	<0.015
Control P B	2 mo	1.360
Control M S	2 mo	0.483
Control T H	3 mo	1.275
Control R A	4 mo	0.460
Mother	24 y	0.097
Father	37 y	0.148
Brother	3 y	0.144
Sister	7 y	0.211
Control P A	2 y	0.372
Control P N	3 y	0.346
Control G S	6 y	0.319
Control C N	27 y	0.375
Control V L	28 y	0.394
Control A D	39 y	0.574
Control H P	67 y	0.305

5 weeks old and 10 days after dietary treatment had started revealed bilateral cataracts localized mainly in the posterior cortex of the lenses. Routine laboratory examinations of blood and urine gave normal results except the test for reducing substance in the urine (clinitest) which was strongly positive. Glucosuria tested with clinitest was absent on repeated examinations. Blood sugar (4 hours after a meal) was 206 mg per 100 ml when determined as reducing substance and 63 mg per 100 ml when determined with glucose oxidase. When galactose was assayed with galactose oxidase a concentration of 178 mg per 100 ml was found. Serum bilirubin concentration, transaminase and lactate dehydrogenase activities and thymol flocculation test were normal when examined both at 4 weeks and at 7 weeks of age. Alkaline phosphatase was increased (45 U) at 4 weeks and within normal limits for age (22 U) at 7 weeks. Serum protein including electrophoretic separation was normal as was the urinary excretion of amino acids. An EEG recorded when the boy was 5 weeks old was judged as borderline because of the presence of some activity with low frequency and occasionally with questionable sharp wave form.

When the boy was 3 1/2 weeks old Milkotal® was replaced by a galactose free formula Sobee®. His previously good general condition improved further in that he took this formula more eagerly than Milkotal®. His weight gain during the last week on Milkotal® was 60 g and during the first week on

Sobee® 300 g. The galactose free diet has been continued and the boy's general condition has remained excellent: he grows and develops normally. The ophthalmological examination was repeated when he was 5 months old. A marked improvement was then found as the opacities had diminished and now consisted only of a thin layer in the posterior cortex; the eye ground could be clearly seen and was normal.

## SPECIAL INVESTIGATIONS

**Galactose in urine.** The boy's urine gave a strongly positive reaction with a galactose specific test paper (6).

For the quantitative assay of galactose in urine we used galactose dehydrogenase (D galactose NAD oxidoreductase EC 1.1.1.48) (Boehringer und Soehne GmbH, Mannheim, Germany) in the following way: to 100 µl of urine (no pre-treatment necessary) was added 1 000 µl of 100 mM TRIS-HCl buffer pH 8.6 and 50 µl of NAD solution (10 mg per ml). After a stable reading at 340 mμ had been obtained 5 µl of galactose dehydrogenase (crystal suspension 5 mg per ml) was added, and the contents of the cuvette was well mixed by stirring with a plastic rod. Reading was then made intermittently until a new plateau had been reached (9-27-33). The amount of galactose was calculated from the increase in optical density. If the increase was larger than 1 000 a new assay was performed with a diluted urine sample. The galactose concentration, determined with this method when the boy was 3 weeks old was found to be 2 550 mg per 100 ml urine.

Earlier urine samples were not available for analysis, since galactose test paper screening of urine is not performed in the hospital where he was born and his mild early symptoms caused no concern and gave no clue to a diagnosis. After the introduction of galactose free diet the galactose concentration rapidly decreased and after 1 week it was less than 4 mg per 100 ml urine.

If galactose in urine is assayed with galactose oxidase interfering substances first must be removed by ion exchange filtration (30).

**Galactitol in urine** Galactitol in urine was assayed in the same way as in a previous investigation (8). In the sample obtained before introduction of galactose free diet there was 340 mg per 100 ml urine and in the sample obtained 1 week later 104 mg per 100 ml urine. Thus when the patient is on a galactose free diet the urinary excretion of galactitol seems to persist longer than that of galactose.

**Galactose 1 phosphate uridylyltransferase activity in red blood cells** As a preliminary test for the demonstration of galactose 1 phosphate uridylyltransferase activity we used the fluorescent spot test of Beutler & Baluda (2). A normal reaction was obtained indicating the presence of galactose 1 phosphate uridylyltransferase. For the quantitative assay of galactose-1 phosphate uridylyltransferase activity we used the method of Tolstrup (3, 32) which is based on UDPG consumption in the presence of galactose 1 phosphate (1). The hemolysate was preincubated with dithiothreitol which activates the enzyme (8, 24). The transferase activity was calculated as units ( $\mu$ moles of UDPG consumed per hour) per g hemoglobin. The activity found was 35.6 units per g hemoglobin which is a normal activity (it is even in the upper normal range since our normal value is  $26.9 \pm 4.5$  (S.D.) units per g hemoglobin). In patients with the classical form of hereditary galactosemia this enzyme is absent.

**Galactokinase activity in red blood cells** The galactokinase activity in red blood cells was measured with the method of Mayes & Guthrie (22) which is a modification of that originally described by Sherman (19). Heparinized venous blood was used.

The blood samples were stored at 0–4°C for a maximum of 24 hours before analysis due to the limited stability of the enzyme (22). Control blood samples were treated in exactly the same way as those from the patient and his relatives. The red blood cells were washed with cold saline and then hemolyzed by freezing and thawing just before the incubation. The amount of radioactive galactose phosphorylated during the reaction and consequently adsorbed onto

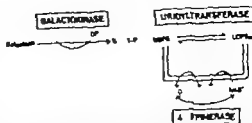


Fig. 1 In the normal subject exogenous galactose is converted into glucose by a reaction sequence catalyzed by three separate enzymes. The deficiency of either one of these enzymes will lead to a state of galactosemia with its own biochemical and clinical characteristics.

ion exchange paper was measured in a Packard liquid scintillation counter (35). The galactokinase activity was calculated as units ( $\mu$ moles of galactose phosphorylated per hour) per ml packed red blood cells. The activity found in the samples from the patient was essentially zero when compared with the activity found in the controls (Table 1). A very weak residual activity less than a few per cent of the normal could be detected. This has been seen also in the other patients with galactokinase deficiency (Grzelmann personal communication). It seems more likely that this residual activity is caused by a weak unspecific effect of some other enzyme than by a small residue of the enzyme in which the patient is deficient.

Normally the galactokinase activity is higher in early infancy than later in life (25) and therefore controls of different ages have been included in Table 1. It is seen that both the mother and the father of the patient have values for galactokinase activity which are only about the half of the normal which clearly fits with the concept that both parents are heterozygotes for the disease. Also the brother appears to be a heterozygote whereas the sister has an intermediate value which does not permit any sure conclusion as to whether she is a heterozygote or not.

**Galactose 1 phosphate in erythrocytes** In the classical form of hereditary galactosemia caused by galactose 1 phosphate uridylyltransferase deficiency galactose 1 phosphate is accumulated in the erythrocytes on galactose in

take. In patients with hereditary galactokinase deficiency this should not be expected to occur since the enzyme catalyzing the formation of galactose 1-phosphate is missing. In accord with this we did not find any galactose 1-phosphate when we analysed with a fluorimetric method (7) washed erythrocytes obtained shortly after the introduction of galactose free diet. Neither was any galactose 1-phosphate found after that the erythrocytes had been preincubated with galactose (150 mg per 100 ml) at 37 C for 30 min.

### DISCUSSION

Theoretically a lack of either one of 3 different enzymes could produce galactosemia, each enzyme deficiency causing its own clinical and biochemical abnormalities (Fig. 1). The lack of UDPGalactose 4 epimerase has so far not been demonstrated. Normal epimerase activity has been found in patients with galactose 1-phosphate uridylyltransferase deficiency (21) and in patients with galactokinase deficiency (14, 31), it was not examined in our patient. There is no reason to suppose a genetic coupling between the epimerase and the other 2 enzymes. If epimerase deficiency occurs, it would be expected to occur as an isolated defect.

There is however reason to believe that a fetus with epimerase deficiency may not be viable. Epimerase transforms UDPGlucose into UDPGalactose, which is the immediate precursor for the galactose part of the complex galactose-containing molecules necessary for the building of the central nervous system. This enzyme is thus involved in a vital process. The two other enzymes do not participate in this endogenous galactose formation.

A lack of galactose-1 phosphate uridylyltransferase causes the classical form of hereditary galactosemia. This disease has an acute onset with the appearance of serious symptoms: vomiting, weight loss, jaundice and convulsions within a few days after milk feeding has started. Large amounts of galactose and galactitol are excreted in the urine and marked aminoacid

uria, due to liver and kidney damage (8), is common. If the patient survives but still receives galactose-containing food he will develop cataracts, mental retardation and liver cirrhosis.

The syndrome caused by galactokinase deficiency is milder. In spite of an urinary excretion of galactose and galactitol which is of the same magnitude as in galactose-1 phosphate uridylyltransferase deficiency, acute clinical symptoms are absent. Reported adult patients have shown cataracts but neither mental retardation nor liver involvement. The lack of early clinical symptoms was predicted by Gitzelman (14) after his study of the first adult patients and was confirmed first by Thalhammer, Gitzelman & Pantlitschko (31) and now by us.

The difference between the two syndromes concerning the presence of acute clinical symptoms may have a biochemical explanation. In galactose 1 phosphate uridylyltransferase deficiency the administration of dietary galactose or lactose leads to the accumulation of galactose 1 phosphate in the tissues, while in galactokinase deficiency galactose 1 phosphate can not be formed from exogenous galactose. It therefore seems to be the sugar phosphate rather than free galactose or the sugar alcohol which causes the toxic symptoms from liver, kidneys and central nervous system. This has an interesting parallel in two disturbances of the metabolism of fructose. In *fructose intolerance* (4) caused by the deficiency of liver aldolase (5, 12, 19) the administration of fructose leads to immediate clinical symptoms (vomiting, tremor, convulsions). In this disease fructose can be phosphorylated but then not further metabolized. In *essential fructosuria* (first described by Zimmer (36)) there is a deficiency of another enzyme necessary for the metabolism of exogenous fructose, namely fructokinase (5, 19, 23, 28). In the latter defect fructose cannot be phosphorylated. Although the administration of fructose leads to at least equally marked excretion of fructose with the urine as in fructose intolerance, there are no clinical symptoms whatsoever. Apparently the tissue accumulation to unmetabolized hexose phosphates is not

galactose or fructose has a pronounced disturbing effect while a high concentration of hexose as such has milder if any metabolic effects.

The one important clinical defect caused by galactokinase deficiency the cataracts also has a biochemical explanation. Galactitol can be isolated from the lens and other tissues of rats which have been fed high galactose diet leading to cataracts (18, 26) and it is likely that deposition of the sugar alcohol is responsible for the opacity of the lens. Both in galactose 1 phosphate uridylyltransferase deficiency (8, 34) and in galactokinase deficiency (13, 14)—also verified in the present case—considerable amounts of galactitol are excreted in the urine. The formation of galactitol from galactose is catalyzed by the enzyme aldose reductase which does not require previous phosphorylation of the substrate and thus offers an alternative pathway when the ordinary metabolism of galactose is blocked. The tissue distribution of aldose reductase is not known (14, 17) but it is known to be present in the lens (16).

Although the metabolism of administered galactose mainly occurs in the liver the diagnosis of galactokinase deficiency as well as that of galactose 1 phosphate uridylyltransferase deficiency is based on the assay of enzymes in the erythrocytes. In both cases the enzyme in red blood cells appears to be controlled by the same gene as the enzyme in the liver. Like the galactose 1 phosphate uridylyltransferase deficiency the galactokinase deficiency is recessively inherited, and the heterozygotes can be identified on the finding of enzyme activity reduced to roughly half of the normal value (15). Both parents of our patient had a reduced enzyme activity a finding to be expected as both of them must be heterozygotes. Besides the patient's brother and possibly also his sister appear to be heterozygotes.

The incidence of reduced enzyme activity was found to be about 1 in 100 when examined in an unselected group of American individuals (22). Provided the same frequency is found in a larger population the incidence of galactosemia due to galactokinase deficiency

can be estimated to roughly 1 in 40 000 newborn infants.

The most serious sequela of the disorder apparently is the cataract. Galactokinase deficiency should be kept in mind as a possible cause of cataracts in infancy and childhood when no other explanation is apparent. In our case opacities of the lenses were already present in the 5 weeks old infant who had received galactose containing food for altogether  $3\frac{1}{2}$  weeks. A considerable improvement was noted 4 months later during this time the infant received only galactose free food. It is thus desirable to detect the metabolic defect and start dietary treatment as early as possible and long before the cataracts have given obvious clinical symptoms. As no symptoms precede the cataract the attainment of this goal means the introduction of a screening procedure of all newborns. All screening methods for hereditary galactosemia which are based on the demonstration of increased blood or urinary galactose concentration will detect also galactokinase deficiency as in fact happened in our patient. The increased blood galactose concentration persists only for a limited period after a meal since there is a rapid urinary excretion plus some metabolism catalyzed by galactose dehydrogenase in the liver and possibly also by aldose reductase (31) a source of error which must not be neglected when blood galactose is used as the screening test for the various forms of galactosemia. This factor may explain why in a screening of 327 000 newborn babies no case of galactokinase deficiency was found (Guttime 1966 cited by Thalhammer et al (31)). Urinary screening may be less dependent on the interval between feeding and sampling but has the disadvantage that in a newborn a urine sample is more difficult to obtain than a blood sample.

In screening for galactose 1 phosphate uridylyltransferase deficiency a simple fluorescence spot test for the enzyme activity in blood cells has been described (2). This test has the advantage that it is independent of dietary galactose (lactose) administration and thus can be performed on cord blood immediately after

birth. Since this test is specific for galactose 1 phosphate uridylyltransferase it will, however, not detect galactokinase deficiency and our patient also showed a normal spot test reaction. If screening for defects in galactose metabolism is based on this kind of tests, therefore a separate test will have to be performed for galactokinase. We made attempts to perform a spot test for galactokinase deficiency by replacing the galactose-1 phosphate, used as substrate in the uridylyltransferase spot test with galactose plus ATP but these attempts were not fruitful since an unspecific fluorescence developed in the presence of ATP also if galactose was excluded. This fluorescence is most probably due to the formation and oxidation of glucose-6-phosphate when ATP is added.

### SUMMARY

A case of hereditary galactokinase deficiency is reported occurring in a newborn boy and detected through routine screening of newborns for increased blood concentration of galactose. Symptoms were equivocal and consisted only of mild feeding difficulties and a slightly insufficient weight gain. The only abnormality found on clinical examination was lens opacities which were obvious when the boy was 5 weeks old. The biochemical abnormalities found were excretion of large amounts of galactose and galactitol in the urine, galactosemia, and essentially no galactokinase activity in the red blood cells. On a galactose free diet, introduced when the boy was 3 1/2 weeks old he grew and developed normally, his cataracts had improved considerably after 4 months. The boy's both parents, one brother and possibly one sister were considered carriers of the autosomal recessive gene, as the enzyme activity found in their red blood cells was roughly half of the normal value.

The clinical findings in patients with the various enzyme defects leading to galactosemia are described and related to the biochemical abnormalities caused by the defects. Possible screening methods are discussed.

### ACKNOWLEDGMENT

Grants which have been obtained from the Sempér Nutrition Foundation, the Majblomman Foundation and the Swedish Medical Research Council (B70-13P 2262 and B70-13A 157) are gratefully acknowledged. Skilful technical assistance has been given by Miss Margareta Lindell and Mrs Ragnita Nörén.

### REFERENCES

- Anderson E P, Katcher H M, Kurenbach A & Iselbacher H J. A specific enzymatic assay for the diagnosis of congenital galactosemia. *J Lab Clin Med* 50: 469 1957.
- Beutler E & Baluda M C. A simple spot screening test for galactosemia. *J Lab Clin Med* 68: 137 1966.
- Brundt N J. *Galaktose 1 (uridyl) transferase. Hereditær galaktosemi, levercirrhose og diabetes*. Dissertation. Munksgaard Forlag, Copenhagen 1966.
- Chambers R A & Pratt R T C. Idiocyrosis to fructose. *Lancet* II: 340 1956.
- Corabiat M & Schwartz R. *Disorders of carbohydrate metabolism in infancy*. Saunders, Philadelphia and London 1966.
- Dahlqvist A. Test paper for galactose in urine. *Scand J Clin Lab Invest* 22: 87 1968.
- To be published 1970.
- Dahlqvist A, Jagenburg R & Mark A. A patient with hereditary galactosemia studied with a screening method for galactose in urine. *Acta Paediatr Scand* 58: 237 1969.
- Doudoroff M. In: *Galactose dehydrogenase of Pseudomonas aeruginosa* in S P Colowick & N O Kaplan (eds) *Methods in enzymology* vol V. Academic Press, New York and London 1962.
- Fantoni G. Hochgradige Galaktose Intoleranz (Galaktose Diabetes) bei einem Kinde mit Neurofibromatose. *Recklinghausen Jb Kinderheilk* 138: 1 1933.
- Die angeborenen Störungen des Kohlenhydratstoffwechsels in H Oitz & F Schmid (eds) *Handbuch der Kinderheilkunde* vol IV Stoffwechsel Ernährung und Verdauung. Springer Verlag, Berlin Heidelberg and New York 1965.
- Frosch E R, Wolf H P, Bartsch H, Prader A & Labhart A. Hereditary fructose intolerance. An inborn defect of hepatic fructose 1-phosphate splitting. *adulose*. *Amer J Med* 34: 151 1963.
- Gitzelmann R. Deficiency of erythrocyte galactokinase in a patient with galactose diabetes. *Lancet* II: 670 1965.
- Hereditary galactokinase deficiency, a newly recognized cause of juvenile cataracts. *Pediatr Res* 1: 14 1967.
- Galactokinase deficiency in D Y Y Hsu (ed) *Galactosemia*. C C Thomas Publisher, Springfield 1969.
- Hayman S & Knochel J H. Isolation and

- properties of lens aldolase reductase *J Biol Chem* 240 877 1965
- 17 Hers H G. L-aldose reductase *Biochim Biophys Acta* 37 120 1960
  - 18 van Heyningen R. Formation of polyols by the lens of the rat with sugar cataract *Nature* 184 194 1959
  - 19 Hsu D Y Y. Inborn errors of metabolism part 1. Chemical aspects. Year Book Medical Publishers Chicago 1966
  - 20 Imelfacher K, J. Anderson E P, Kurahashi A. & Kalcker H M. Congenital galactosemia: a single enzymatic block in galactose metabolism *Science* 123 635 1956
  - 21 Kalcker H M, J. Anderson E P & Imelfacher K. J. Galactosemia: a congenital defect in a nucleotide transferase *Biochim Biophys Acta* 20 282 1956
  - 22 Mayes J S & Guthrie R. Detection of heterozygotes for galactokinase deficiency in a human population *Biochem Genet* 2 219 1964
  - 23 Marks V & Samols E. Glucosamines other than diabetes mellitus. In F Dickens P J Randle & W J Whelan (eds). *Carbohydrate metabolism and its disorders* vol 2. Academic Press London and New York 1968
  - 24 Malfon W J & Fedesco T A. An improved assay of erythrocyte and leukocyte galactose 1-phosphate uridylyl transferase: stabilization of the enzyme by a thiol protective reagent *J Lab Clin Med* 66 980 1965
  - 25 NG W G, Donnell G N & Bergren W R. Galactokinase activity in human erythrocytes of individuals of different ages *J Lab Clin Med* 66 115 1965
  - 26 Quan Ma R. & Wells W W. The distribution of galactitol in tissues of rats fed galactose *Biochem Biophys Res Commun* 30 486 1965
  - 27 Rinaldi H & Hollmann S. Bestimmungs-methode von D-Glucose und niedermolekularen Kohlenhydraten. III. D-Galactose. In H Bartelmeier W Heyde & W Thore (eds). *D-Glucose und verwandte Verbindungen in Medizin und Biologie*. Ferdinand Enke Verlag Stuttgart 1966
  - 28 Schapira F, Schapira E & Dryfus J C. La liaison enzymatique de la fructose bovine. Enzyme *Biol Clin (Basel)* 1 170 1962
  - 29 Sherman J R. Rapid enzyme assay technique utilizing radioactive substrate ion-exchange paper and liquid scintillation counting *Anal Biochem* 5 548 1963
  - 30 Tengstrom H. Enzymatic determination of glucose and galactose in urine *Scand J Clin Lab Invest* 18 Suppl. 92 104 1966
  - 31 Thallhammer O, Gitzelmann R. & Panitschko M. Hypergalactosemia and galactosuria due to galactokinase deficiency in a newborn *Pediatr Acta* 42 441 1964
  - 32 Tostrup N. *Hereditær galaktosemi. Galaktose 1-fosfat-uridylyltransferase mangel med hereditær polydiuresis og arvegang*. Dissertation. Arne Friis Hansen Forlag Copenhagen 1965
  - 33 Wallenfels K. & Kurz H. Über die Spezifität der Galaktosedehydrogenase und deren Anwendung als analytisches Hilfsmittel *Biochem Z* 335 559 1962
  - 34 Wells W W, Pittman T A & Egge T J. The isolation and identification of galactitol from the urine of patients with galactosemia *J Biol Chem* 239 3192 1964
  - 35 Wilson D B & Hogness D S. Galactokinase and uridine diphosphogalactose 4-epimerase from *Escherichia coli*. In S P Colowick & N O Kaplan (eds). *Methods in enzymology* vol VIII. Academic Press New York and London 1966
  - 36 Zimner K. Lavallose im Harn eines Diabetikers *Deutsch Med Wochr* 2 328 1876

Submitted Febr 20 1970

Accepted April 20 1970

(A. D.) Chemical Research Department  
E blocket  
Larsretat  
220 81 Lund  
Sweden

**Key words:** hereditary galactokinase deficiency; galactosemia; infants; cataract

## C REACTIVE PROTEIN IN SERUM FROM INFANTS AS DETERMINED WITH IMMUNODIFFUSION TECHNIQUES

### II *Infants with Various Infections*

J SAXSTAD L. A NILSSON and L. A HANSON

*From the Department of Paediatrics and the Departments of Bacteriology and Immunology, Institute of Medical Microbiology, University of Göteborg, Göteborg, Sweden*

Several authors have shown that infants are able to form C reactive protein (CRP) during the course of various infectious diseases (1-5). In these investigations employing the capillary tube precipitation method, the diagnostic usefulness of CRP in small infants has been suggested.

With the more accurate and sensitive immunodiffusion methods CRP has been detected in 17% of infants apparently healthy on clinical examination (6). Usually it was present only in trace amounts; however, the same techniques have now been applied in a study of the occurrence of CRP in infants hospitalized because of various infections.

### MATERIAL AND METHODS

Serum samples were obtained from 113 infants 1-15 months of age admitted to the hospital because of an infectious disease. The samples were obtained within 10 days of the onset of the disease in these patients. Nineteen were diagnosed as having acute pyelonephritis caused by Gram-negative bacteria. From a few of the patients with recurrent attacks of pyelonephritis consecutive blood samples were obtained. Thirty were diagnosed as gastroenteritis, 33 as bronchitis or bronchopneumonia, and 23 as upper respiratory infection or otitis. In the latter group the presence or absence of bacterial pathogens in throat cultures was recorded. Finally, 8 patients were included with evidence of viral infections (2 with serous meningitis, 4 with exanthema subitum and 2 with

ECHO infections). The sera were tested within 24 hours of sampling.

The CRP determinations were performed with the comparative double diffusion in gel technique and the single radial immunodiffusion method as earlier described (4). Concentrations of 0.5-1.0  $\mu\text{g/ml}$  of CRP are recorded as trace amounts and higher concentrations are expressed in  $\mu\text{g/ml}$ .

### RESULTS

No significant relationship was seen between the age of the infants and the CRP concentrations found (Fig. 1a). On the other hand a correlation was observed between the CRP concentrations and the sedimentation rates of the blood samples (Fig. 1b).

CRP was found in all sera from infants with pyelonephritis, often in high amounts (Fig. 2a and Table 1). This was seen both in patients with an acute onset of disease and in those where an infection was discovered by positive urinary cultures on a routine check-up. CRP was studied in consecutive serum samples from some patients. A rapid rise to a high CRP concentration after onset of infection was seen as exemplified by patient H. M. in Fig. 3. After treatment of the infection the CRP concentration decreased quickly below demonstrable quantities as shown by patient A. E. in the same figure. This patient again had a mi-

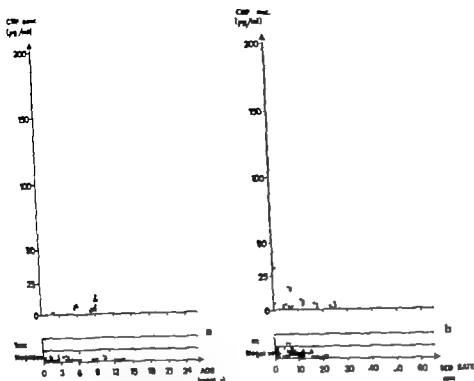


Fig. 1 (a) Relation between CRP concentration and age in 113 infected infants (b) Relation between CRP concentration and sedimentation rate in 113 infants

nor temporary rise in CRP concentration with out any clinical or laboratory signs of recurrent infection. But at 14 weeks a symptomatic recurrent attack of pyelonephritis was accompanied by high CRP concentrations.

In the infants with gastroenteritis CRP could not be detected in 19 of the 30 patients where as it was present in trace amounts in two and in rather low concentrations in the remaining nine (Fig. 2 b).

In the 33 infants with bronchitis or bronchopneumonia, CRP was demonstrable in all but 7 patients (Fig. 2 c and Table 1).

CRP was found in all of the 7 patients with otitis and 12 of the 16 patients with acute pharyngitis (Fig. 2 d and Table 1). In the combined otitis-pharyngitis group 12 patients had pathogenic bacteria (*Hemophilus influenzae*, *Streptococcus*, *Staphylococcus*, *β hemolytic streptococcus* and *coliform organisms*) cultured from their throats. These 12 patients had CRP in their

sera. In contrast the 4 patients without CRP had ordinary throat cultures (Table 2).

Finally in the small group of 8 patients with evidence of a viral infection CRP was detected in five instances (Fig. 2 e and Table 1).

## DISCUSSION

It has been suggested that CRP determinations could be of diagnostic help especially for infectious diseases in early infancy (1-6). Using the capillary tube precipitation technique Felix et al. (1) found that CRP appeared in sera from two-thirds of infected infants in contrast to their normal controls where CRP was found in serum in a frequency of 27%.

In this study quantitative determinations of CRP were performed with immunodiffusion methods which are known to be more sensitive, specific and accurate than the generally employed capillary tube precipitation method.



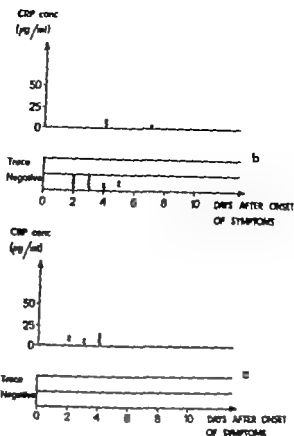
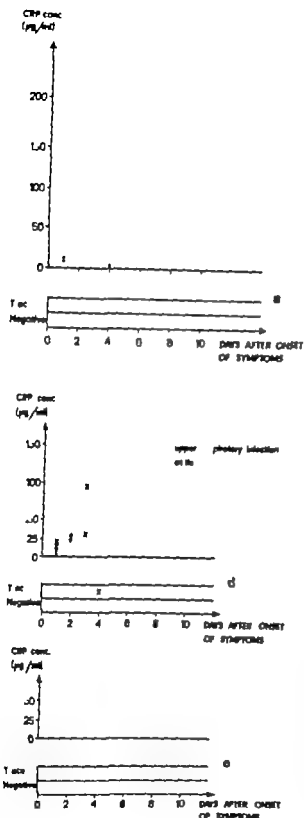


Fig 2 Serum content of CRP in (a) 19 infants with pyelonephritis (b) 30 with gastroenteritis (c) 33 with bronchitis or bronchopneumonia (d) 23 with pharyngitis or otitis and (e) 8 with viral infections. The lower part of each figure shows those samples which had only traces of CRP or were negative on analysis with the double diffusion method. Those positive with this method were quantitated by the single radial diffusion technique as is depicted in the diagram above.

(4) Using these techniques CRP was demonstrated in amounts of  $>1 \mu\text{g}/\text{ml}$  in sera from about two thirds of infected infants. With the same methods CRP was found in concentrations exceeding  $1 \mu\text{g}/\text{ml}$  in only 4% of apparently healthy infants. (6) During the course of

infections such as gastroenteritis it was observed in only one third of the cases whereas it was seen in all cases of pyelonephritis. The reason for such variations may be differences in the severity and localization of the infections as well as in causative microorganisms. It is sug-

Table 1 CRP in serum in relation to various infectious diseases

Diagnosis	No of patients	C reactive protein (CRP)		
		Present	Trace	Not demonstrable
Pyelonephritis	19	18	1	0
Gastroenteritis	30	9	2	19
Bronchitis or broncho-pneumonia	33	24	2	7
Diarrhoea or pharyngitis	23	17	2	4
Viral infections	8			
serous otitis media	2	0	0	2
scarlet fever	4	2	1	1
ECHO infection	2	2	0	0
Total	113	72	8	33

gestive that all the patients with pyelonephritis caused by Gram negative bacteria as well as those with upper respiratory infections with bacterial pathogens isolated in throat cultures had demonstrable CRP. In the small group of 8 patients with presumed viral infections CRP was not detectable in three. This as well as the low frequency of CRP appearing among the patients with gastroenteritis presumably mostly of viral etiology may support the earlier observation of several authors that bacterial infections more often induce the formation of CRP than do viral infections (3).

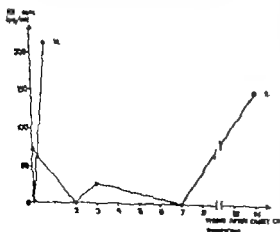


Fig. 5 CRP content in consecutive serum samples from patients with pyelonephritis

Table 2 CRP in serum related to throat culture findings in children with otitis or pharyngitis

	CRP demonstrable	CRP not demonstrable
Bacterial pathogens in throat cultures	12	0
Ordinary throat culture	7	4

Studies of consecutive samples from a few patients with recurrent attacks of pyelonephritis indicated that the CRP concentration rapidly increased to high values after the onset of infection and decreased after adequate treatment. This indicates that CRP may be a good index of the course of pyelonephritis infection.

These findings suggest that in infants with certain types of infections especially bacterial infections quantitative CRP determination can be of help for diagnosis and follow up. It seems worthwhile to evaluate further the relation of CRP to the course of pyelonephritis.

## SUMMARY

Quantitative CRP determinations using immunodiffusion methods have been performed on 113 infants with various infections. Occurrence and concentration of CRP differed for various infectious diseases. Thus it was found in only a third of the infants with gastroenteritis whereas it was found in all infants with pyelonephritis mostly in high concentrations.

## REFERENCES

1. Felix N. S., Nakajima H. & Kagan B. Serum C reactive protein in infections during the first six months of life. *Pediatrics* 37: 270, 1966.
2. Hansen L. A. & Nilsson L. A. Studies in C reactive protein during the pre and neonatal period. *Acta Path. Microbiol. Scand.* 56: 409, 1962.
3. Hedlund P. Clinical and experimental studies on C reactive protein (acute phase protein). *Acta Med. Scand. Suppl.* 361, 1961, p. 71.
4. Nilsson L. A. Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum. *Acta Path. Microbiol. Scand.* 72: 129, 1968.

- 5 Philipson L. & Tveten B. C reactive protein in infancy. Its appearance during the first year of life, transplacental passage and electrophoretic pattern. *Acta Paediat Scand* 46: 1 1957
- 6 Saxstad J, Nilsson L. A. & Hanson L. A. C reactive protein in serum from infants as determined with immunodiffusion techniques. I Healthy infants. *Acta Paediat Scand* 59: 25 1970

Submitted Febr 16 1970

Accepted March 26 1970

(L. A. H.) Dept of Immunology  
Inst of Medical Microbiology  
413 46 Goteborg  
Sweden

## A DOUBLE BLIND STUDY OF THE EFFECT OF PHENOBARBITONE ON NEONATAL HYPERBILIRUBINAEMIA AND FREQUENCY OF EXCHANGE TRANSFUSION

M VEST E SIGNER E. WEISSEK and A. OLAFSSON

*From the Children's Hospital University of Basle Basle Switzerland*

It has been observed that infants of mothers treated with phenobarbitone because of epilepsy or preeclampsia had a lower incidence of serum bilirubin levels above 10 mg/100 ml than controls. A less clearcut effect was seen when phenobarbitone injections were given to the infants after birth (9). In a more detailed study the administration to pregnant women, the infants only or to both mothers and infants was compared (10). Best results were obtained again when the mother was treated for at least a fortnight before delivery but treating the infants by a total of 10 injections gave closely comparable results. This effect of phenobarbitone therapy in neonatal jaundice has been confirmed (5, 6). The treatment of the infant alone however was less effective than treating the mother (5). Young & Field (13) in Hongkong not only saw an effect on serum bilirubin but also on the frequency of exchange transfusions needed because of high serum bilirubin. When treatment was started after neonatal jaundice had appeared phenobarbitone did not seem to have an effect (3).

In premature infants few studies seem to have been done. In a retrospective analysis no significant difference between infants of mothers who had or had not received barbiturates during pregnancy could be demonstrated (12). With Nulchamand orally no difference in the frequency of exchange transfusion was found

although serum bilirubin was lower in the treated group on the 6th day (4).

Because the premature infants are those most likely to develop dangerous hyperbilirubinaemia which necessitates exchange transfusion, it seemed indicated to study the influence of phenobarbitone on serum bilirubin concentration and frequency of exchange transfusion especially in this age group.

### PATIENTS AND METHODS

Newborns admitted to the children's hospital within 24 hours after delivery because of prematurity respiratory difficulties or other reasons were randomly assigned to either the treated or the control group. Neither to the physician nor the nurses was it known whether an infant was receiving placebo or phenobarbitone.

A total of 10 injections of phenobarbitone or placebo solution was given spaced 8 hours apart. The first injection was given as soon as possible after admission. The single dose injected was the following: for newborns less than 1 000 g body weight 0.1 ml (1 mg) between 1 000 and 1 500 g 0.2 ml (2 mg) between 1 500 and 2 000 g 0.3 ml (3 mg) and so on to a maximum of 0.5 ml (5 mg) for infants weighing 2 501 g or more. The total dose per 24 hours varied therefore between 3 mg and 15 mg of phenobarbitone.

Serum bilirubin concentration was measured daily for 10 days and less frequently red cells haemoglobin haematocrit reticulocytes prothrombin time, urea and blood pH was estimated. Every child was carefully observed for side effects by one of us (E. S.).

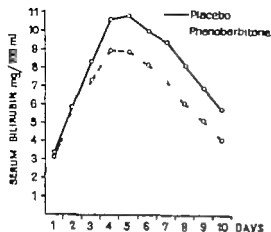


Fig 1 Serum bilirubin levels in control and phenobarbitone treated infants (Mean values of all infants)

140 infants were assigned to the study 70 in each group. Cases with Rh or ABO incompatibility and critically ill infants were excluded from the study.

## RESULTS

From the 140 infants 10 in the treated and 8 in the control group died with one exception within 3 days after birth. The difference in deaths in the two groups is not significant by the  $\chi^2$  test. Four infants dropped from the treated and 2 from the control group for various reasons. Thus there were 56 newborns in the barbiturate and 60 in the placebo group. Of these 43 respectively 46 had a body weight of less than 2500 g. In the whole group the mean weight was 2070 g in the treated and

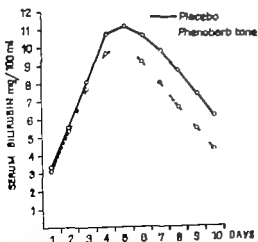


Fig 2 Serum bilirubin levels in control and phenobarbitone treated premature infants

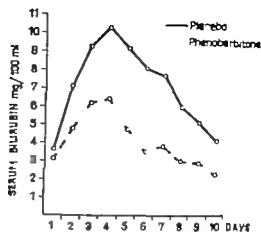


Fig 3 Serum bilirubin levels in control and phenobarbitone treated full term infants

2203 g in the control group. The mean weight of the infants with less than 2500 g body weight was 1805 g (treated) and 1872 g (controls). Mean gestational age was 44 days before term in the former and 47.5 days in the latter group.

**Frequency of exchange transfusions.** There were 11 exchange transfusions in the placebo and 1 in the treated group. This difference by the  $\chi^2$  test is significant ( $p < 0.01$ ). All exchange transfusions with one exception had to be performed in infants of less than 2500 g.

**Serum bilirubin concentration.** The course of serum bilirubin of the two groups is shown in Fig 1. The placebo group has significantly higher values from the fourth day on. (In the  $t$  test  $p < 0.025$  on day 7 to 9  $p < 0.01$ ). If one regards premature and full term infants separately the difference in bilirubin concentration in the treated and control group is more prominent in the latter. In the premature group this difference is significant at a 5% level on the 7th and at a 1% level between the 8th and 10th day (Fig 2). In the small group of full term infants (13 and 14 infants)  $p$  is lower than 0.05 on the third and fourth day and lower than 0.025 on the 5th and 6th day (Fig 3).

In contrast to the difference in bilirubin levels red cell count haemoglobin reticulocytes haematocrit prothrombin urea and blood pH were identical in both groups.

## DISCUSSION

The present investigation indicates that in pre mature infants the number of exchange transfusions can be decreased significantly by treatment with small doses of phenobarbitone after delivery. This confirms the results of Yeung & Field (13). The doses used were always lower than the amount of 6 to 7 mg/per kg/24 h given to newborn infants as a sedative and far below that given in convulsions.

The average serumbilirubin levels are also lower in the treated infants. The decrease is more pronounced in fullterm than in premature infants. This has also been observed by Ramboer et al. (6). The rather low dose used in the lowest weight classes (only 3 mg/24 h in infants less than 1 000 g) might contribute to this result.

When one considers the definite mortality of exchange transfusion especially in low weight premature with other disturbances e.g. respiratory distress syndrome phenobarbitone treatment seems to offer an alternative. Against this a possible danger of the drug therapy has to be carefully balanced.

As has been mentioned a daily detailed clinical examination was performed without knowing whether an infant belonged to the placebo or treated group. The results were recorded. Evaluation of these notes showed that there was no discernible effect of phenobarbitone on general behaviour, motor activity, feeding, respiration, reflexes etc. This was also the experience of Trofic (10). The measurement of blood pH,  $P_{CO_2}$  and standard bicarbonate showed no ill effects. In view of the fact, that phenobarbitone has been used for decades in newborn infants for a variety of reasons and in higher dosage we feel that untoward effects of the small doses used during only 3 days are unlikely.

Trofic (10) has investigated whether the albumin binding of unconjugated bilirubin is decreased by the phenobarbitone. No competing was found. It is therefore improbable that the observed decrease of serum bilirubin is related to an alteration of protein binding. In addition

Valaes & Doxadis (11) demonstrated that the faecal excretion of bile pigment is increased by phenobarbitone.

It has been shown that phenobarbitone and other substances induce the synthesis of hepatic microsomal enzymes including glucuronyl transferase (2, 7). An enhancement not only of bilirubin conjugation but also of biliary excretion by phenobarbitone has been shown in animals (1, 8). A similar mechanism might also cause the lower concentration and quicker decrease of serum bilirubin in infants.

## SUMMARY

In a double blind study the effect of phenobarbitone on neonatal hyperbilirubinaemia and the frequency of exchange transfusion was investigated. Newborn infants admitted were randomly assigned to one of two groups. The treated group comprised 56 and the control group 60 infants. Either placebo solution or phenobarbitone was injected 3 m at 8 hourly intervals for a total of 10 injections. The single dose varied between 1 mg and 5 mg according to weight. One exchange transfusion had to be performed in the treated and 11 in the control group. This difference is statistically significant. Serumbilirubin concentrations from the 4th day on were significantly lower in the treated infants than in controls.

## REFERENCES

1. Cize, C. & Yaffe, S. J. Barbiturate enhancement of bilirubin conjugation and excretion in young and adult animals. *Pediatr Res* 2: 361, 1963.
2. Conney, A. H. Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev* 19: 317, 1969.
3. Cunningham, M. D., Macle, J. W. & Peters, E. R. Clinical experience with phenobarbitone in icterus neonatorum. *Lancet* i: 550, 1969.
4. Kuntzel, H. W. & Henkel, G. A. Der Einfluss von Nikotinamid auf den Serumbilirubin Spiegel des Frühgeborenen. *Munch. Kinderheilk* 117: 84, 1969.
5. Maurer, A. M., Wolff, J. A., Finster, M., Popper, F. J., Finkbein, E., Kosterman, R. & Conney, A. H. Reduction in concentration of total

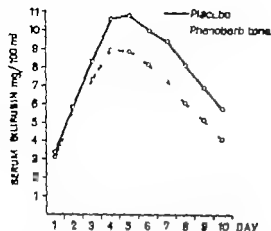


Fig 1 Serum bilirubin levels in control and phenobarbitone treated infants (Mean values of all infants)

140 infants were assigned to the study 70 in each group. Cases with Rh or ABO incompatibility and critically ill infants were excluded from the study.

## RESULTS

From the 140 infants 10 in the treated and 8 in the control group died with one exception within 3 days after birth. The difference in deaths in the two groups is not significant by the  $\chi^2$  test. Four infants dropped from the treated and 2 from the control group for various reasons. Thus there were 56 newborns in the barbiturate and 60 in the placebo group. Of these 43 respectively 46 had a body weight of less than 2500 g. In the whole group the mean weight was 2070 g in the treated and

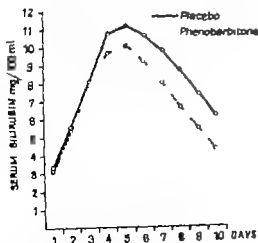


Fig 2 Serum bilirubin levels in control and phenobarbitone treated premature infants

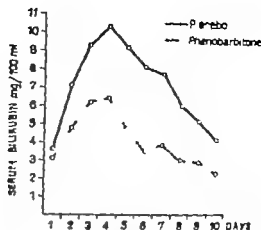


Fig 3 Serum bilirubin levels in control and phenobarbitone treated full term infants

2203 g in the control group. The mean weight of the infants with less than 2500 g body weight was 1805 g (treated) and 1872 g (controls). Mean gestational age was 44 days before term in the former and 47.5 days in the latter group.

**Frequency of exchange transfusions.** There were 11 exchange transfusions in the placebo and 1 in the treated group. This difference by the  $\chi^2$  test is significant ( $p < 0.01$ ). All exchange transfusions with one exception had to be performed in infants of less than 2500 g.

**Serum bilirubin concentration.** The course of serum bilirubin of the two groups is shown in Fig 1. The placebo group has significantly higher values from the fourth day on (In the  $t$  test  $p < 0.025$  on day 7 to 9  $p < 0.01$ ). If one regards premature and full term infants separately, the difference in bilirubin concentration in the treated and control group is more prominent in the latter. In the premature group this difference is significant at a 5% level on the 7th and at a 1% level between the 8th and 10th day (Fig 2). In the small group of full term infants (13 and 14 infants)  $p$  is lower than 0.05 on the third and fourth day and lower than 0.025 on the 5th and 6th day (Fig 3).

In contrast to the difference in bilirubin levels, red cell count, haemoglobin, reticulocytes, haematocrit, prothrombin, urea and blood pH were identical in both groups.

## SUDDEN UNEXPECTED DEATH IN INFANCY

*An Autopsy Study*

H STALSBERG

*From the Department of Pathology Ullevål Hospital  
University of Oslo Oslo Norway*

The etiology and pathogenesis of sudden and unexpected death in apparently healthy infants (SUD) is still an unsolved problem. A variety of pathogenetic mechanisms have been suggested (7-8, 16) among which are status thymico-lymphaticus, accidental suffocation by bedclothes, anaphylactic reaction to cow's milk (12, 13) and hyperacute bacterial or viral infections. All of the proposed hypotheses have weak points either from evidence that is in direct opposition to the hypothesis or from the lack of supportive evidence. The thymus is not larger in SUD than in other cases of sudden death (9), the composition of the inspired air is not significantly altered under the cover of ordinary bedclothes (17), serum protein studies in SUD victims have given no evidence for the presence of allergy to cow's milk (10, 13, 14, 15), microbiological post mortem examinations have usually failed to show pathogenic organisms in the majority of cases (2, 6, 15).

The SUD cases autopsied at Ullevål hospital over a 10-year period have been reviewed. For comparison sudden deaths from non-infectious causes and deaths from acute infections in the same age group have been included. The aim has been to fit the autopsy findings in SUD into a spectrum of findings in cases of explained deaths thus seeking evidence for the nature of SUD.

## MATERIAL AND METHODS

During the 10-year period 1959-1968, 207 infants between 2 weeks and 2 years of age were autopsied at Ullevål hospital. From anamnestic data, 53 of these cases were selected for the present study. The material was divided into five diagnostic groups for which abbreviated designations will be used.

SUD: sudden unexpected death, 17 boys and 9 girls. The group comprises infants in whom no sign of illness except trivial symptoms of common cold had been noted prior to death and in whom autopsy findings were inconclusive.

ACC: accidents and other sudden deaths from non-infectious causes, 6 boys and 2 girls. Five infants died from suffocation, one died after 3 hours from a head injury, one died from acute pontine and cerebellar hemorrhage, one died suddenly from endocardial fibroelastosis.

SDI: sudden and unexpected death during an apparently mild infectious disease other than common cold, 4 boys and no girls. All four infants had suffered from acute gastroenteritis, which had lasted for 1, 1, 2 and 5 days respectively. None of the infants in this group had been considered to be seriously ill by their surroundings or had been seen by a doctor until they were found dead.

RDI: rapid death from infectious disease with less than 2 days duration of symptoms, 3 boys and 2 girls. Three infants died from pneumonia or acute tracheobronchitis, one died from shock and convulsions, one was believed to have died from septicemia. All had high fever.

DI: death from infectious disease with 2 to 14 days duration of symptoms, 5 boys and 5 girls. Five infants died from acute gastroenteritis after 2, 3, 3, 4 and 9 days, four died from bronchopneumonia after 2, 4, 5 and 9 days, one died from acute myocarditis and a probably viral pneumonia after 7 days of illness.

Anamnestic data were taken from the clinical records, and macroscopic findings were taken from the autopsy reports.



serum bilirubin in offspring of woman treated with phenobarbitone during pregnancy *Lancet* II 122 1968

6 Ramboer C Thompson R P H & Williams R Controlled trials of phenobarbitone therapy in neonatal jaundice *Lancet* I 966 1969

7 Remmer H Die Induktion arzneimittelabbauender Enzyme im endoplasmatischen Reticulum der Leberzelle durch Pharmaka *Dtsch med Wschr* 92 2001 1967

8 Roberts R J & Plas G L Effect of phenobarbital on the excretion of an exogenous bilirubin load *Biochem Pharmacol* 16 827 1967

9 Trolle D Phenobarbitone and neonatal icterus *Lancet* I 251 1968

10 — Decrease of total serum bilirubin concentration in newborn infants after phenobarbitone treatment *Lancet* II 705 1968

11 Valaes T & Doxiadis N A Trial of maternal phenobarbitone medication in a population with high unspecific neonatal hyperbilirubinaemia *Abstr Ann Meeting Europ Soc Pediat Res Inter-laken* Sept 14-18 1969

12 Walker W Hughes M I & Barton M Barbiturate and hyperbilirubinaemia of prematurity *Lancet* I 548 1969

13 Yeung C Y & Field C E Phenobarbitone therapy in neonatal hyperbilirubinaemia *Lancet* II 135 1969

Submitted April 6 1970  
Accepted May 8 1970

Children's Hospital  
Romergasse 8  
CH-4000 Basle  
Switzerland

Key words Neonatal jaundice exchange transfusion Phenobarbitone therapy enzyme induction

nation aspirated material was seen in alveoli or small bronchi in only 3 cases of SUD and in 1 case in each of the ACC SDI and DI groups.

Desquamation of the bronchiolar epithelium which has been taken to indicate acute viral infection (2) was roughly estimated as the percentage of bronchiolar epithelium that had been separated from the basement membrane and the percentage of bronchiolar epithelium that had been fragmented into single cells or small groups of a few cells each. The results give no evidence for an association between desquamation of bronchiolar epithelium and SUD (Table 4).

Also the following features occurred with about the same frequency in all groups. Hyperemia and increased mucous secretion in the trachea and main bronchi, erythrocytes inflammatory cells and edematous fluid in the lumen of small bronchi, macrophages in the alveoli, peribronchovascular lymphocyte aggregates. No case of cytomegalovirus was diagnosed.

Acute bronchiolitis was seen in 2 cases in the SUD group and in 1 case in each of the groups SDI, RDI and DI.

Focal collapse of the lungs had been de-

Table 6 Lung hyperemia microscopic evaluation

	SUD	ACC	SDI	RDI	DI
Absent	4	2	2	—	4
Slight or moderate	9	3	1	3	4
Severe	12	3	—	2	2
Total	25	8	3	5	10

scribed from macroscopic examination in about half of the SUD cases and in only 2 cases in other groups. By microscopic examination, however, partial diffuse or focal collapse was a common finding in all groups.

Lung edema tended to be more pronounced in SUD cases than in the other groups (Table 5) and a similar though weaker tendency was seen for hyperemia of the lungs (Table 6).

Occasional intraalveolar granulocytes were seen in eight SUD infants. No more than occasional intraalveolar granulocytes were also seen in 2 cases of the RDI group, one of which had clinical signs of pneumonia and died 20 hours after the onset of symptoms, the second RDI case was diagnosed as acute tracheobronchitis and died 30 hours after the onset of symptoms. On the other hand a few intraalveolar granulocytes were also seen in 2 cases of the ACC group.

Table 4 Desquamation of bronchiolar epithelium. Means of individual estimates

	SUD (°)	ACC (°)	SDI (°)	RDI (°)	DI (°)
Portion of epithelium separated from the basement membrane	62	64	57	49	64
Portion of epithelium fragmented into single cells or small cell groups	44	33	40	21	41

Table 5 Lung edema microscopic evaluation

	SUD	ACC	SDI	RDI	DI
Absent	4	3	3	2	5
Slight or moderate	10	4	—	4	4
Severe	12	1	—	1	1
Total	25	8	3	3	10

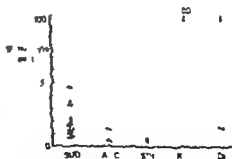


Fig. 2 Maximum number of granulocytes per 1/4 visual field (40x objective, 10x field of view) of lung parenchyma. SUD: sudden unexpected and unexplained deaths; ACC: accidents and other explained non-infectious sudden deaths; SDI: sudden unexpected deaths during acute gastroenteritis; RDI: deaths from infectious diseases of less than 2 days duration; DI: deaths from infectious diseases of 2-14 days duration. The asterisks indicate 2 cases of bronchopneumonia with abscess formation and granulocyte fragmentation, in which no counts were made.

Table 1 Age distribution

Age in months	SUD	ACC	SDI	RDI	DI
1/2-4	13	5	1	5	5
4-8	7	1	—	—	1
8-12	4	1	—	—	2
12-24	2	1	3	—	2
Total	26	8	4	5	10

Table 2 Seasonal variation of deaths

	SUD	ACC	SDI	RDI	DI
Jan-March	8	3	—	1	4
Apr-June	5	1	3	1	2
July-Sept	4	3	1	—	1
Oct-Dec	9	1	—	3	3
Total	26	8	4	5	10

The available histological sections from heart (39 cases) kidney (46 cases) lung (51 cases) thymus (42 cases) and spleen or lymph nodes (32 cases) were reviewed. The examination of sections was made without knowledge of which diagnostic group each specimen belonged to, and all histological findings were recorded according to a pre-made list of relevant details. In the lung sections the maximum number of granulocytes per 1/4 visual field of alveolar tissue (40× objective 10× hpl Zeiss eye piece) was estimated. The granulocytes were counted in two or three fields selected for high cellularity and the highest count obtained in each case was recorded. In the thymus sections the ratio between cortical and medullary areas was estimated. Each section was projected with a magnification of 16× onto a sheet of paper and areas of cortex and medulla were drawn and measured with a planimeter.

### ANAMNESTIC DATA

The age distribution is shown in Table 1. Most cases of SUD occur during the first 4 months of life, and only 2 cases were seen during the second year.

Seasonal variation is similar for SUD and for deaths from acute infections with alarming symptoms (RDI, DI) both occurring with high frequency during winter months (Table 2).

Ten of the SUD infants had shown trivial symptoms of upper respiratory tract infections in the days prior to death, and three more had been more restless than usual.

Thirteen SUD infants were said to have been found with bedclothes covering their faces. There was a striking difference in age between those who had their faces covered and the rest of the SUD group (Table 3). No infant younger than 3 months of age was said to have had its face covered, whereas nearly all SUD infants older than 4 months of age were said to have had their faces covered.

Nutrition is known in only seven SUD infants, six had been artificially fed and one had been at least partially nourished by the mother.

### AUTOPSY FINDINGS

**Heart.** There was 1 case of acute myocarditis in the DI group, 1 case of endocardial fibroclastosis in the ACC group and 1 case of antrum-ventriculare commune in a mongoloid infant in the RDI group. The remaining cases had no sign of heart disease or abnormality.

**Kidneys.** Fibrosis and degeneration of scattered glomeruli have been described in SUD (3/15) were seen in all groups and were no more common in SUD than in infants that died from known causes.

**Respiratory organs.** Petechial hemorrhages in the lungs and other thoracic viscera were seen in some cases of all groups. Microscopic petechiae on chest organs were even more frequent in the ACC group (7 of 8) than in the SUD group (15 of 26).

Aspiration of ventricular contents to the respiratory passages appeared not to be an important factor in SUD. By microscopic exami-

Table 3 Covering of the face and age distribution in SUD

	Ages in months/days						
Infants said to have had their faces covered	3/0	3/17	4/28	5/18	6/19	6/21	6/28
	6/23	7/9	8/23	9/5	10/4	10/28	13/30
Infants with no mentioning of covering of the face	0/16	1/6	1/22	1/29	2/4	2/8	2/10
	2/10	2/27	3/3	3/9	3/16	4/15	19/26

among different groups. Nor are varying degrees of lung hyperemia responsible for the variation of granulocyte counts. Four SUD infants with no hyperemia of the lungs had  $17 \pm 11$  granulocytes (mean  $\pm 1$  S.D.) nine SUD infants with slight or moderate hyperemia had  $26 \pm 22$  granulocytes and twelve SUD infants with severe hyperemia had  $25 \pm 19$  granulocytes per  $1/4$  visual field.

For comparison with the findings in the lungs the number of granulocytes in ten randomly chosen renal glomeruli was counted in each case of the SUD, ACC and RDI groups in which kidney sections were available (35 cases). Contrary to the findings in the lungs the average numbers of glomerular granulocytes showed no obvious difference among the three groups.

Colonies of bacteria were seen in the lung parenchyma in seven SUD cases, one of which had microscopic evidence of aspiration. In the other groups colonies of bacteria were seen in one SDI case in which microscopic evidence of aspiration was also present in two DI cases with fully developed bronchopneumonia and in one DI case with a possible imminent pneumonia. No colonies of bacteria were seen in ACC and RDI cases. Bacteriological cultures were made from only two of the SUD cases in which bacterial colonies were seen in the histological sections; both showed the presence of *Staphylococcus aureus*. Bacteriological examination from the lungs of two other SUD cases showed *E. coli* in one and normal bronchial flora in the other.

**Thymus.** The mean weight of the thymus was slightly higher in the ACC group (33 g) than in the SUD group (26 g) but the difference is not statistically significant. The ratio of cortical areas to medullary areas in thymus sections was practically identical in ACC and SUD groups. In the SDI, RDI and DI groups both the weight and the cortico-medullary ratio tended to be lower although in most cases in which the illness had lasted for less than 2 days the thymus parameters were within the range of the ACC and SUD cases.

**Spleen and lymph nodes.** Neither spleen weight nor the number and size of germinal centers of spleen and lymph nodes or their content of fragmented cell nuclei showed any significant difference among the groups.

## DISCUSSION

None of the findings in this material could justify definite conclusions about the causal mechanisms of SUD. As in other reports (2-9) aspiration of ventricular contents to the respiratory passages appear to be a possible mechanism only in a small minority of cases. When seen together several of the present observations could be taken to indicate that bacterial pneumonias that reach only to the initial phase of the disease could be the cause of many cases of SUD either as the single determinant factor or possibly in conjunction with other factors predisposing to sudden death.

Most remarkable is the finding of high numbers of neutrophilic granulocytes in the alveolar septa in the older SUD infants and the relation between granulocyte numbers and age in the SUD group. The observations may mean that the defense mechanisms in SUD infants under 4 months of age are so poorly developed that virulent infections lead to death before the infants have been able to mobilize their granulocyte reaction whereas after the age of 4 months this reaction is gradually strengthened until at the end of the first year the defense mechanisms are usually good enough to prevent the occurrence of SUD. In this connection it may be noticed that the maximum SUD frequency at 3 months of age (6) coincides with the age at which serum concentrations of IgG immunoglobulins reach minimum levels (1, 4).

It is very rare that older children or adults die during the first day of a bacterial pneumonia but when this occurs the picture is different from that commonly diagnosed as pneumonia by the pathologist. In the first day of a lobar pneumococcal pneumonia hyperemia and edema are the main features and only a

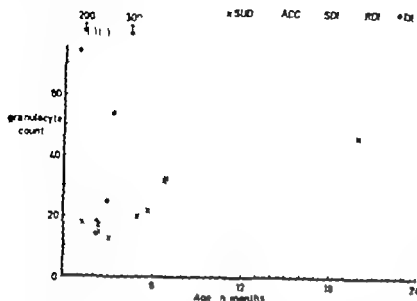


Fig. 2 Same data as in Fig. 1 related to the age of the infants. Abbreviations and statistics as in Fig. 1.

Granulocytes in the alveolar septa were much more common than intravascular granulocytes, the great majority being neutrophils. Fig. 1 shows the counted maximum numbers of granulocytes both intravascular and septal, per 1/4 visual field of lung parenchyma. The normal number that is the number of granulocytes that can be expected when no lung disease is present can best be estimated from the findings in the infants who died from obvious accidents. With the exception of one infant the granulocyte counts of the ACC group are below twenty. The one exception is an infant whose allocation to the ACC group or to the SUD group was questionable. Thus normal granulocyte counts are tentatively taken to be below twenty per 1/4 visual field.

In the SUD group granulocyte counts range from 3 to 68 and in ten SUD cases the counts are higher than 20. In the groups of recognized infectious diseases (SDI, RDI, DI) all cases that had signs or symptoms of respiratory infections except for the case of probable viral pneumonia in the DI group had granulocyte counts higher than 20. This includes 1 case of gastroenteritis with 65 granulocytes in the SDI group, in which a fibrinous pleuritis was also present. A second case of gastroenteritis in the DI group had a granulocyte count of 45. No further evidence of respiratory infection is known in this case but the presence of

an imminent complicating pneumonia remains a possible explanation. In the RDI group the granulocyte counts above 20 were 25 in an infant with clinical sepsis, 30 in an infant with marked acute bronchiolitis and tracheobronchitis, 54 in a mongoloid infant with osseous atroventricular commune and clinical signs of pneumonia, about 200 in an infant with fully developed bronchopneumonia. Granulocyte counts below 20 were recorded in the SDI group in 2 cases of gastroenteritis, in the DI group in 4 cases of gastroenteritis and in the RDI group in 1 case with extensive lung hemorrhage.

There is an obvious association between age and granulocyte counts in the SUD group as shown in Fig. 2. The granulocyte counts are low and less than 20 in all SUD infants younger than 4 months of age. In older SUD infants there is a gradual increase of granulocyte counts with age. No similar association between age and granulocyte counts is evident in the other groups. It should be recalled that the ACC case with the highest granulocyte count had a doubtful group allocation and might possibly have been grouped with the SUD cases.

There is no consistent association between granulocyte counts and resuscitation, either within the SUD group or in the comparison

- 1 Parsh W E, Hall L W & Coombs R R A The effect of anaesthesia on anaphylaxis in Gunn rats *Immunology* 6 462 1963
- 12 Parsh W E, Richards C B, France N E & Coombs R R A Further investigations on the hypothesis that some cases of cot-death are due to a modified anaphylactic reaction to cow's milk *Int Arch Allergy* 24 215 1964
- 13 Pritson R D A & Good R A Antibodies to cow's milk proteins — their presence and significance *Pediatrics* 31 209 1963
- 14 Stowers D, Callahan E L & Clay J Sudden unexpected death in infancy. A new hypothesis of cause *Clin Pediatr (Phila)* 5 243 1966
- 15 Valdes-Dapena, M A Sudden and unexpected death in infancy. A review of the world literature 1954 1966 *Pediatrics* 39 123 1967
- 17 Woolley P V Jr Mechanical suffocation during infancy. A comment on its relation to the total problem of sudden death *J Pediatr* 26 572 1945
- 18 Wright G, Payling & Heard B E The lungs including the trachea and bronchi and the pleura in G Payling Wright and W St C Symmers (eds) *Systemic pathology* Longmans, London 1966 p 333

Submitted March 24 1970

Accepted June 2 1970

Dept of Pathology

Ullevål sykehus

Oslo 1

Norway

**Key words** Sudden death pathology bacterial pneumonia

few erythrocytes and granulocytes are present in the alveolar fluid. It is not until the second day that granulocytes appear in appreciable numbers in the alveoli (11-18). Hyperemia and edema of the lungs were prominent in most of the SUD cases in the present material and a few intraalveolar granulocytes were present in one third. Similar findings were made in some of the infants who died after a very brief but recognized history of clinical pneumonia or acute tracheobronchitis.

Other evidence for the role of infections in SUD are the frequent observation of bacterial colonies in histological lung sections in this material, and the high frequency of preceding symptoms of upper respiratory tract infections and the seasonal variation parallel to that of respiratory infections as have been common findings in other studies as well (2, 5-6-9).

The reason why pathogenic bacteria have been demonstrated so infrequently in most series of SUD autopsies may lie in the conditions under which bacteriological cultures have been taken and grown. Johnstone & Lawry (10) paid special attention to this point, and by setting up cultures from bronchial secretions under a set of different culture conditions they were able to demonstrate the presence of pathogenic bacteria in 37 out of 55 cases of SUD.

Covering of the face with bedclothes was the rule in SUD infants older than 4 months and was absent from SUD infants younger than 3 months in the present study. A possible explanation is that under a feeling of cold that accompanies a rapid rise in body temperature, covering of the face is actively sought for by those infants who are old enough to be able to do so. Thus, covering of the face with bedclothes may not be a cause of death but rather a side effect of a disease that leads to SUD.

### SUMMARY

Autopsy findings in 26 typical cases of sudden and unexpected infant death (SUD) are reported and compared to findings in non-infectious sudden deaths and deaths from acute

infectious diseases. Lung edema and hyperemia were on the average more pronounced in SUD than in the controls. Bacterial colonies in histological lung sections were seen in seven SUD cases. In the control groups such findings were generally associated either with bronchopneumonia or with microscopic evidence of aspiration. Increased numbers of neutrophilic granulocytes were seen in the lung alveolar septa in SUD infants over 4 months of age, and occasional intraalveolar granulocytes were present in one third of the SUD cases. Covering of the face with bedclothes was the rule in SUD infants older than 4 months and was absent from SUD infants younger than 3 months of age. It is suggested that bacterial pneumonias that reach only to the initial phase of the disease may be the cause of many cases of SUD and that covering of the face with bedclothes may be actively sought for by the older SUD infants under a feeling of cold accompanying a rapid rise in body temperature.

### REFERENCES

- 1 Allansmith M R, McClellan B H & Butterworth M. Individual patterns of immunoglobulin development in ten infants. *J Pediatr* 75: 1231-1969.
- 2 Banks A L. An enquiry into sudden death in infancy. *Monthly Bull. Minut Health* (London) 17: 182-1958.
- 3 Barrett A M. Sudden death in infancy. In D. Gaudner (ed.) *Recent advances in paediatrics*. J. & A. Churchill Ltd. London 1954. p. 301.
- 4 Berg T. The immunoglobulin development during the first year of life. A longitudinal study. *Acta Paediat Scand* 58: 229-1969.
- 5 Cooke R T & Welch E G. A study in cot death. *Brit Med J* 2: 1549-1964.
- 6 Geertinger P. *Plutselig uventet spædbarnsdød*. København: Munksgaard, København 1966.
- 7 — Plutselig uventet spædbarnsdød. *Läkertidningen* 66: 677-1969.
- 8 Giersten J C. Plutselig död hos spæbarn. *Nord Med* 77: 825-1967.
- 9 Jacobsen T & Voigt J. Sudden and unexpected infant death II. Result of medico-legal autopsies of 356 infants aged 0-2 years. *Acta Med Leg Soc (Læge)* 9: 133-1958.
- 10 Johnstone J M & Lawry H S. Role of infection in cot deaths. *Brit Med J* 1: 706-1966.
- 11 Millard M. Lung pleura and mediastinum in W. A. D. Anderson (ed.) *Pathology* 5th ed. Mosby St. Louis 1966. p. 686.

- 12 Parsh W F, Hall L W & Coombs R R A. The effect of anaesthesia on anaphylaxis in Guinea pigs *Immunology* 6 462 1963
- 13 Parsh W E, Richards C B, Francis N E & Coombs R R A. Further investigations on the hypothesis that some cases of cot-death are due to a modified anaphylactic reaction to cow's milk *Int Arch Allerg* 24 215 1964
- 14 Peterson R D A & Good R A. Antibodies to cow's milk proteins — their presence and significance *Pediatrics* 31 209 1963
- 15 Stowers E, Callahan E L & Clay J. Sudden unexpected death in infancy. A new hypothesis of cause *Clin Pediatr (Phila)* 5 243 1966
- 16 Valdes-Dapena M A. Sudden and unexpected death in infancy. A review of the world literature 1954-1966 *Pediatrics* 39 123 1967
- 17 Woolley P V Jr. Mechanical suffocation during infancy. A comment on its relation to the total problem of sudden death *J Pediatr* 26 572 1945
- 18 Wright, G Payling & Heard, B E. The lungs including the trachea and bronchi and the pleura in G Payling Wright and W St C Symmers (eds) *Systemic pathology* Longmans, London 1966 p 333

Submitted March 24 1970

Accepted June 2 1970

Dept of Pathology

Ullevål sykehus

Oslo 1

Norway

**Key words** Sudden death pathology bacterial pneumonia



## THE ELECTROLYTE AND PROTEIN CONTENTS AND OUTPUTS IN DUODENAL JUICE AFTER PANCREOZYMIN AND SECRETIN STIMULATION IN NORMAL CHILDREN AND IN PATIENTS WITH CYSTIC FIBROSIS<sup>1</sup>

G ZOPPI D H SHMERLING<sup>2</sup> D GABURRO and A PRADER

From the Clinica Paediatrica University of Ferrara Ferrara Italy and the  
Kinderspital University of Zurich Zurich Switzerland

The concentration of electrolytes and protein in duodenal juice of various animals and in man is well known (1, 2, 7, 8, 9, 11, 14, 17, 18). Yet systematic studies on total outputs and secretion patterns of ions and protein after hormonal stimulation with pancreozymin and secretin are lacking especially so in infants and children (5, 6).

This problem assumes a particular importance in disorders of exocrine pancreatic secretion, e.g. in cystic fibrosis of the pancreas (6) since in this disease a generalized disturbance of water and electrolyte movement in exocrine tissues and particularly in the exocrine pancreas has been recently assumed to play an important role (6, 10).

In order to study such a hypothesis duodenal contents have to be sampled quantitatively as possible after hormonal stimulation of the exocrine pancreas, so that outputs and secretion rates can be calculated. This was attempted in the investigations reported here.

### MATERIAL AND METHODS

#### Subjects studied

17 children aged 9 months to 13 years have been studied. 12 did not present any evidence of exo-

crine pancreatic insufficiency. 5 suffered from cystic fibrosis (CF) of the pancreas as demonstrated by elevated sweat electrolytes, pulmonary involvement and steatorrhea.

#### Methods

**Duodenal intubation.** A triple lumen double balloon tube was placed in the duodenum under fluoroscopic control as described previously in detail (5, 18). Pancreozymin and secretin (Boots) were given intravenously (2 IU/kg body weight each) and the duodenal content sampled in 10 min periods twice after pancreozymin and three times after secretin.

**Laboratory procedures.** Immediately after sampling the volume and pH of each fraction were measured and bicarbonate concentration determined according to Lagerlöf (12). Sodium and potassium were assayed by flame spectrophotometry, total calcium according to Diehl et al. (4) and magnesium as described by Thiers (15). Protein was estimated by the method of Lowry et al. (13).

Protease, lipase and amylase activities of duodenal juice were also tested as described previously (5) as control of pancreatic activity. Electrolytes were assayed and reported here only in patients in whom at least some pancreatic enzyme activity was detectable. Cases with no enzyme activities were excluded.

Fluid volume, enzyme activities, protein and electrolytes were expressed as total outputs per kg body weight and per 50 min after hormonal stimulation. Secretion rates of fluid volume, protein and electrolytes expressed per kg and per min were calculated separately for the post-pancreozymin and the post-secretin periods.

### RESULTS

Table 1 shows the total outputs of enzyme activities both in controls and in patients ex-

<sup>1</sup> Reported in part at the annual Meeting of the European Society of Pediatric Gastroenterology, Interlaken, Switzerland, September 1969.

<sup>2</sup> Supported by the Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (Grant No. 4344).

Table 1 Total outputs of peptidases  $\alpha$  amylase and lipase in duodenal juice of normal controls and patients with cystic fibrosis of the pancreas (CF) (mean and range/kg body weight 50 min after pancreozymin and secretin stimulation)

Case	Chymotrypsin ( $\mu$ g)	Trypsin ( $\mu$ g)	Carboxypeptidase A (I U 10 <sup>3</sup> )	$\alpha$ Amylase (I U)	Lipase (I U)
Controls					
n=12					
Mean	880	765	712	651	1464
(range)	(270-1900)	(215-2000)	(185-2200)	(160-2150)	(350-5000)
Patients					
1	0	0	0	28	0
2	0	2	0.5	6	41
3	0	0	0	220	0
4	16	41	85	73	341
5	0	0	0	8	0

prised per kg and per 50 min after hormonal stimulation

In Table 2 total outputs of fluid volume protein and electrolytes per kg and per 50 min after hormonal stimulation in normal controls and in patients are reported. The values of fluid volume and electrolytes appear to be strongly lowered in CF patients. As previously described (18) protein output is also decreased but the difference is not statistically significant. Expressed as percentages of the normal controls (Table 2) the secretion of fluid volume sodium potassium and calcium in CF patients is reduced to the same amount. Bicarbonate is

reduced much more whereas magnesium and protein much less than the fluid volume.

Table 3 shows the secretion rates of fluid volume (expressed in ml per kg and per min) of protein and of electrolytes ( $\mu$ g per kg and per min) in normal controls and in patients after pancreozymin and Table 4 those after secretin stimulation. A comparison of the secretion rates after pancreozymin and after secretin in normal controls (Fig. 1) shows that the maximal secretion rate of sodium and bicarbonate is reached after secretin whereas that of protein calcium and magnesium is reached after pancreozymin. Secretion rates of potassium and

Table 2 Total outputs of fluid volume protein and electrolytes in duodenal juice of normal controls and patients with cystic fibrosis of the pancreas (CF) (mean  $\pm$  1 S.D. ml/kg body weight 50 min after pancreozymin and secretin stimulation)

	Normal controls	CF patients	CF patients in % of normals
n	1	5	
Volume (ml/kg/50 min)	3.9 $\pm$ 1.6	0.8 $\pm$ 0.4	20
Protein	15.4 $\pm$ 5.5	9.7 $\pm$ 8.6	63
Bicarbonate	15 $\pm$ 7	1.4 $\pm$ 1.5	9
Sodium	13.9 $\pm$ 6.4	2.5 $\pm$ 0.8	18
Potassium	0.8 $\pm$ 0.3	0.3 $\pm$ 0.07	25
Calcium	0.1 $\pm$ 0.05	0.04 $\pm$ 0.01	33
Magnesium	0.05 $\pm$ 0.01	0.03 $\pm$ 0.02	60

Table 3 Secretion rates of fluid volume protein and electrolytes in normal controls and in patients with cystic fibrosis of the pancreas (CF) (mean  $\pm$  1 S.D.  $\mu$ g/kg body weight 1 min after pancreozymin stimulation)

	Normal controls	CF patients	CF patients in % of normals
n	12	5	
Volume (ml/kg/min)	0.06 $\pm$ 0.01	0.02 $\pm$ 0.004	33
Protein	579 $\pm$ 107	108 $\pm$ 133	35
Bicarbonate	167 $\pm$ 73	35 $\pm$ 24	9
Sodium	240 $\pm$ 101	65 $\pm$ 40	27
Potassium	16.8 $\pm$ 6.5	5.6 $\pm$ 6.4	35
Calcium	3.6 $\pm$ 1.4	0.9 $\pm$ 0.8	25
Magnesium	1.2 $\pm$ 0.3	0.8 $\pm$ 0.7	71

## THE ELECTROLYTE AND PROTEIN CONTENTS AND OUTPUTS IN DUODENAL JUICE AFTER PANCREOZYMIN AND SECRETIN STIMULATION IN NORMAL CHILDREN AND IN PATIENTS WITH CYSTIC FIBROSIS<sup>1</sup>

G ZOPPI B H SHMERLING D GABURRO and A PRADER

*From the Clinica Paediatrica Università di Ferrara Ferrara Italy and the  
Kinderhospital Universität of Zurich Zurich Switzerland*

The concentration of electrolytes and protein in duodenal juice of various animals and in man is well known (1 2, 7 8 9 11 14 17 18). Yet systematic studies on total outputs and secretion patterns of ions and protein after hormonal stimulation with pancreozymin and secretin are lacking especially so in infants and children (5 6).

This problem assumes a particular importance in disorders of exocrine pancreatic secretion, e.g. in cystic fibrosis of the pancreas (6) since in this disease a generalized disturbance of water and electrolyte movement in exocrine tissues and particularly in the exocrine pancreas has been recently assumed to play an important role (6 10).

In order to study such a hypothesis duodenal contents have to be sampled as quantitatively as possible after hormonal stimulation of the exocrine pancreas so that outputs and secretion rates can be calculated. This was attempted in the investigations reported here.

### MATERIAL AND METHODS

#### *Subjects studied*

17 children aged 9 months to 13 years have been studied. 12 did not present any evidence of exo-

crine pancreatic insufficiency. 5 suffered from cystic fibrosis (CF) of the pancreas as demonstrated by elevated sweat electrolytes pulmonary involvement and steatorrhea.

#### *Methods*

**Duodenal intubation.** A triple lumen double balloon tube was placed in the duodenum under fluoroscopic control as described previously in detail (5 18). Pancreozymin and secretin (Boots) were given intravenously (2 IU/kg body weight each) and the duodenal content sampled in 10 min periods twice after pancreozymin and three times after secretin.

**Laboratory procedures.** Immediately after sampling the volume and pH of each fraction were measured and bicarbonate concentration determined according to Lagerlöf (12). Sodium and potassium were assayed by flame spectrophotometry, total calcium according to Diehl et al (4) and magnesium as described by Thiers (15). Protein was estimated by the method of Lowry et al (13).

Proteinase, lipase and  $\alpha$ -amylase activities of duodenal juice were also tested as described previously (5) as control of pancreatic activity. Electrolytes were assayed and reported here only in patients in whom at least some pancreatic enzyme activity was detectable. Cases with no enzyme activities were excluded.

Fluid volume, enzyme activities, protein and electrolytes were expressed as total outputs per kg body weight and per 50 min after hormonal stimulation. Secretion rates of fluid volume, protein and electrolytes expressed per kg and per min were calculated separately for the post pancreozymin and the post secretin periods.

### RESULTS

Table 1 shows the total outputs of enzyme activities both in controls and in patients ex-

<sup>1</sup> Reported in part at the annual Meeting of the European Society of Pediatric Gastroenterology Interlaken Switzerland September 1969.

Supported by the Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (Grant No 4344).

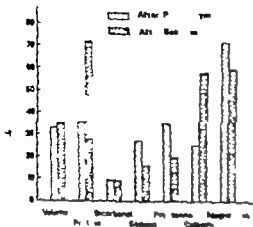


Fig. 2 Comparison of secretion rates after pancreozymin and secretin in cystic fibrosis patients (% of mean values found in normal controls)

are similar. Bicarbonate is reduced much more and protein and magnesium much less than the fluid volume. This means that the concentrations of protein and magnesium in duodenal juice of CF patients after hormonal stimulation is relatively higher than the concentration of sodium and potassium.

The mechanisms of secretion of water and of various electrolytes seem therefore to be altered in different degrees.

#### Secretion rates

In normal controls the secretion rates of fluid volume after pancreozymin and after secretin (Fig. 1) seem not to be significantly different after both hormonal stimuli. Pancreozymin is known to stimulate the acinar cells of the pancreas whereas secretin stimulates the centroacinar and the intercalated duct cells. The secretion patterns of water in both compartments of the gland are therefore probably similar.

The secretion rates of sodium, potassium and magnesium in CF patients was shown to be more strongly reduced after secretin than of calcium and protein after pancreozymin and that of fluid volume and bicarbonate similarly reduced after both hormonal stimulations. In patients with proved cystic fibrosis having exocrine pancreatic insufficiency but some de-

tectable enzymatic activities in the duodenal juice not only the secretin-dependent secretion produced by the centroacinar and the intercalated duct cells is greatly decreased but also the pancreozymin-dependent secretion from the acinar cells.

The non selective destruction of the gland in the disease, which in our patients (all presenting with steatorrhea) must have been quite extensive leads to a disorganized pattern of defects in the secretion rates. There is no evidence for a more accentuated defect in either the pancreozymin or the secretin-dependent phase of secretion. On the other hand there is a non parallel reduction of fluid volume, sodium and bicarbonate after hormonal stimulation in CF patients. Since in exocrine cells the transport of water and sodium is passive while the transport of bicarbonate may be active (10) it seems likely that in cystic fibrosis both the passive and the active transport mechanisms of ions across the cell membrane of the exocrine pancreas are altered.

#### SUMMARY

The total pancreatic outputs and the secretion rates of fluid volume, protein, bicarbonate, sodium, potassium, calcium and magnesium were studied after pancreozymin and secretin stimulation in 12 control children and in 5 patients with cystic fibrosis of the pancreas (CF). Duodenal contents were collected through a double balloon triple lumen rubber tube thus avoiding contamination by gastric juice and distal losses. In CF patients compared to the normal controls decreased outputs of fluid volume, protein and electrolyte were found.

Secretion rates calculated per kg body weight and per min of fluid volume, protein and electrolytes were analyzed separately for the post pancreozymin and the post-secretin periods. In control children the secretion rates of sodium and bicarbonate were higher after secretin stimulation whereas those of protein, calcium and magnesium were higher after pancreozymin stimulation. Secretion rates of

Table 4 Secretion rates of fluid volume protein and electrolytes in normal controls and in patients with cystic fibrosis of the pancreas (CF) (mean  $\pm 1$  SD  $\mu\text{g/kg}$  body weight 1 min after secretin stimulation)

	Normal controls	CF patients	CF patients in of normals
n	12	5	
Volume (ml/kg/min)	0.07 $\pm$ 0.02	0.02 $\pm$ 0.008	28
Protein	262 $\pm$ 143	202 $\pm$ 220	72
Bicarbonate	436 $\pm$ 209	36 $\pm$ 46	9
Sodium	300 $\pm$ 156	48.5 $\pm$ 16.9	16
Potassium	15.3 $\pm$ 5.9	3.1 $\pm$ 0.5	20
Calcium	1.2 $\pm$ 0.8	0.7 $\pm$ 0.3	58
Magnesium	0.8 $\pm$ 0.4	0.5 $\pm$ 0.4	59

fluid volume did not show any significant difference between the post pancreozymin and the post secretin periods.

In CF patients secretion rates of fluid volume protein and electrolytes are lowered. In Fig. 2 secretion rates after pancreozymin and after secretin expressed as percentages of mean values found in normals are compared. The secretion rate of sodium, potassium and magnesium is more strongly reduced after secretin, that of protein and calcium after pancreozymin and that of fluid volume and bicarbonate is similarly reduced after both hormonal stimulations.

## DISCUSSION

### Total Outputs

With the method described above we could determine with a good approximation the total pancreatic outputs of fluid volume, protein and electrolytes after hormonal stimulation with pancreozymin and secretin. The double balloon tube used here—as previously discussed (5, 18)—permits as far as possible a quantitative sampling of duodenal contents without any contamination with gastric juice and distal losses.

Duodenal juice is often bile stained after pancreozymin stimulation since pancreozymin (Boots) shows a cholecystokinin activity which is presumably quite constant. This fact, which is negligible in the case of enzyme determinations, may play a role in the case of electrolytes. Bile contamination would in such a case lead to a similar contribution of electrolytes to the juice after pancreozymin stimulation in both controls and patients. As the electrolyte content of bile is relatively low (3, 6) such a contamination would probably not alter the results significantly.

In the CF patients studied here, the pancreatic secretion was strongly diminished but not completely absent as evidenced by the detectable enzyme activities.

In CF patients the reduction of total outputs of fluid volume, sodium, potassium and calcium

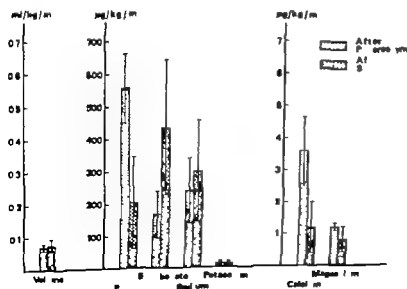


Fig. 1 Comparison of the secretion rates after pancreozymin and secretin in normal controls (secretion/kg body weight/min).

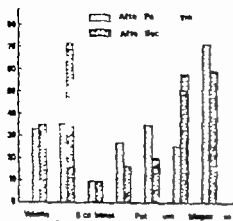


Fig 2 Comparison of secretion rates after pancreozymin and secretin in cystic fibrosis patients (% of these values found in normal controls)

are similar. Bicarbonate is reduced much more and protein and magnesium much less than the fluid volume. This means that the concentrations of protein and magnesium in duodenal juice of CF patients after hormonal stimulation is relatively higher than the concentration of sodium and potassium.

The mechanisms of secretion of water and of various electrolytes seem therefore to be altered in different degrees.

#### Secretion rates

In normal controls the secretion rates of fluid volume after pancreozymin and after secretin (Fig. 1) seem not to be significantly different after both hormonal stimuli. Pancreozymin is known to stimulate the acinar cells of the pancreas whereas secretin stimulates the centroacinar and the intercalated duct cells. The secretion patterns of water in both compartments of the gland are therefore probably similar.

The secretion rates of sodium, potassium and magnesium in CF patients was shown to be more strongly reduced after secretin than of calcium and protein after pancreozymin and that of fluid volume and bicarbonate similarly reduced after both hormonal stimulations. In patients with proved cystic fibrosis having exocrine pancreatic insufficiency but some de-

tectable enzymatic activities in the duodenal juice not only the secretin-dependent secretion produced by the centroacinar and the intercalated duct cells is greatly decreased, but also the pancreozymin-dependent secretion from the acinar cells.

The non selective destruction of the gland in the disease which in our patients (all presenting with steatorrhea) must have been quite extensive leads to a disorganized pattern of defects in the secretion rates. There is no evidence for a more accentuated defect in either the pancreozymin or the secretin-dependent phase of secretion. On the other hand there is a non parallel reduction of fluid volume, sodium and bicarbonate after hormonal stimulation in CF patients. Since in exocrine cells the transport of water and sodium is passive while the transport of bicarbonate may be active (10) it seems likely that in cystic fibrosis both the passive and the active transport mechanisms of ions across the cell membrane of the exocrine pancreas are altered.

#### SUMMARY

The total pancreatic outputs and the secretion rates of fluid volume, protein, bicarbonate, sodium, potassium, calcium and magnesium were studied after pancreozymin and secretin stimulation in 12 control children and in 5 patients with cystic fibrosis of the pancreas (CF). Duodenal contents were collected through a double balloon triple lumen rubber tube thus avoiding contamination by gastric juice and distal losses. In CF patients compared to the normal controls decreased outputs of fluid volume, protein and electrolytes were found.

Secretion rates calculated per kg body weight and per min of fluid volume, protein and electrolytes were analyzed separately for the post pancreozymin and the post secretin periods. In control children the secretion rates of sodium and bicarbonate were higher after secretin stimulation whereas those of protein, calcium and magnesium were higher after pancreozymin stimulation. Secretion rates of

Table 4 Secretion rates of fluid volume protein and electrolytes in normal controls and in patients with cystic fibrosis of the pancreas (CF) (mean  $\pm 1$  SD  $\mu\text{g/kg body weight 1 min}$  after secretin stimulation)

	Normal controls	CF patients	CF patients in of normals
n	12	5	
Volume (ml/kg/min)	0.07 $\pm$ 0.02	0.02 $\pm$ 0.006	28
Protein	262 $\pm$ 143	202 $\pm$ 220	72
Bicarbonate	436 $\pm$ 209	36 $\pm$ 46	9
Sodium	300 $\pm$ 156	28.5 $\pm$ 16.9	16
Potassium	15.3 $\pm$ 3.9	3.1 $\pm$ 0.5	20
Calcium	1.2 $\pm$ 0.8	0.7 $\pm$ 0.3	58
Magnesium	0.8 $\pm$ 0.4	0.5 $\pm$ 0.4	59

fluid volume did not show any significant difference between the post pancreozymin and the post secretin periods.

In CF patients secretion rates of fluid volume protein and electrolytes are lowered. In Fig. 2, secretion rates after pancreozymin and after secretin expressed as percentages of mean values found in normals are compared. The secretion rate of sodium, potassium and magnesium is more strongly reduced after secretin than that of protein and calcium after pancreozymin and that of fluid volume and bicarbonate is similarly reduced after both hormonal stimulations.

## DISCUSSION

### Total Outputs

With the method described above we could determine with a good approximation the total pancreatic outputs of fluid volume protein and electrolytes after hormonal stimulation with pancreozymin and secretin. The double balloon tube used here—as previously discussed (5, 18)—permits as far as possible a quantitative sampling of duodenal contents without any contamination with gastric juice and distal losses.

Duodenal juice is often bile stained after pancreozymin stimulation, since pancreozymin (Boots) shows a cholecystokin activity which is presumably quite constant. This fact, which is negligible in the case of enzyme determinations, may play a role in the case of electrolytes. Bile contamination would in such a case lead to a similar contribution of electrolytes to the juice after pancreozymin stimulation in both controls and patients. As the electrolyte content of bile is relatively low (3, 6) such a contamination would probably not alter the results significantly.

In the CF patients studied here, the pancreatic secretion was strongly diminished but not completely absent as evidenced by the detectable enzyme activities.

In CF patients the reduction of total outputs of fluid volume sodium potassium and calcium

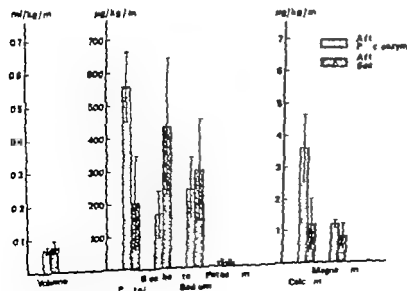


Fig. 1 Comparison of the secretion rates after pancreozymin and secretin in normal controls (secretion/kg body weight/min)

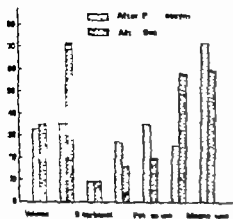


Fig. 2 Comparison of secretion rates after pancreozymin and secretin in cystic fibrosis patients (% of mean values found in normal controls)

are similar. Bicarbonate is reduced much more and protein and magnesium much less than the fluid volume. This means that the concentrations of protein and magnesium in duodenal juice of CF patients after hormonal stimulation is relatively higher than the concentration of sodium and potassium.

The mechanisms of secretion of water and of various electrolytes seem therefore to be altered in different degrees.

#### Secretion rates

In normal controls the secretion rates of fluid volume after pancreozymin and after secretin (Fig. 1) seem not to be significantly different after both hormonal stimuli. Pancreozymin is known to stimulate the acinar cells of the pancreas whereas secretin stimulates the centroacinar and the intercalated duct cells. The secretion patterns of water in both compartments of the gland are therefore probably similar.

The secretion rates of sodium, potassium and magnesium in CF patients was shown to be more strongly reduced after secretin, that of calcium and protein after pancreozymin and that of fluid volume and bicarbonate similarly reduced after both hormonal stimulations. In patients with proved cystic fibrosis having exocrine pancreatic insufficiency but some de-

tectable enzymatic activities in the duodenal juice not only the secretin-dependent secretion produced by the centroacinar and the intercalated duct cells is greatly decreased but also the pancreozymin dependent secretion from the acinar cells.

The non selective destruction of the gland in the disease which in our patients (all presenting with steatorrhea) must have been quite extensive leads to a disorganized pattern of defects in the secretion rates. There is no evidence for a more accentuated defect in either the pancreozymin or the secretin-dependent phase of secretion. On the other hand there is a non parallel reduction of fluid volume, sodium and bicarbonate after hormonal stimulation in CF patients. Since in exocrine cells the transport of water and sodium is passive while the transport of bicarbonate may be active (10) it seems likely that in cystic fibrosis both the passive and the active transport mechanisms of ions across the cell membrane of the exocrine pancreas are altered.

#### SUMMARY

The total pancreatic outputs and the secretion rates of fluid volume, protein, bicarbonate, sodium, potassium, calcium and magnesium were studied after pancreozymin and secretin stimulation in 12 control children and in 5 patients with cystic fibrosis of the pancreas (CF). Duodenal contents were collected through a double balloon triple lumen rubber tube thus avoiding contamination by gastric juice and distal losses. In CF patients compared to the normal controls decreased outputs of fluid volume, protein and electrolytes were found.

Secretion rates calculated per kg body weight and per min of fluid volume, protein and electrolytes were analyzed separately for the post pancreozymin and the post secretin periods. In control children the secretion rates of sodium and bicarbonate were higher after secretin stimulation, whereas those of protein, calcium and magnesium were higher after pancreozymin stimulation. Secretion rates of



fluid volume and potassium did not show any significant difference

In CF patients the secretion rates of sodium, potassium and magnesium were more reduced after secretin whereas those of calcium and protein are more reduced after pancreozymin. The secretion rates of fluid volume and bicarbonate are similarly reduced after both hormonal stimulation

The possible role of a disturbance of the tubular and the renal secretion of the pancreas in the pathogenesis of cystic fibrosis is discussed

## REFERENCES

- 1 Burnbaum D & Hollander F. Relation between bicarbonate concentration and rate of canine pancreatic secretion. *Amer J Physiol* 209: 966 1965
- 2 Case R M, Harper A A & Scratcherd T. The secretion of electrolytes and enzymes by the pancreas of the anesthetized cat. *J Physiol* 201: 335 1969
- 3 Cooke R E & Crowley L G. Replacement of gastric and intestinal fluid losses in surgery. *New Eng J Med* 246: 637 1952
- 4 Drehl H & Ellingboe J L. Indicator for titration of calcium in presence of magnesium using disodium dihydrogen ethylenediamine tetraacetate. *Anal Chem* 28: 882 1956
- 5 Hadorn B, Zoppi G, Shmerling D H, Prider A, McIntyre I & Anderson C M. Quantitative assessment of exocrine pancreatic function in infants and children. *J Pediatr* 73: 39 1968
- 6 Hadorn B, Johansen P G & Anderson C M. Pancreozymin secretin tests of exocrine pancreatic function in cystic fibrosis and the significance of the results for the pathogenesis of the disease. *Canad Med Ass J* 93: 377 1968
- 7 Husky J. Calcium content of duodenal juice. *Amer J Dig Dis* 12: 725 1967
- 8 Henriksen F W. Effects of pancreozymin on the canine pancreatic secretion of fluid and bicarbonate. *Scand J Gastroent* 3: 637 1968
- 9 Henriksen F W & Wormsley H. The relation

between the concentrations of bicarbonate and protein in the pancreatic juice in the dog. *J Physiol* 187: 285 1966

- 10 Johansen P G, Anderson C M & Hadorn B. Cystic fibrosis of the pancreas. A generalized disturbance of water and electrolyte movement in exocrine tissues. *Lancet* 1: 435 1968
- 11 Janowitz H D & Dreiling D A. The pancreatic secretion of fluid and electrolytes. In *The exocrine pancreas: normal and abnormal function*. Ciba Foundation, Churchill London 1962: p 115
- 12 Lagerlöf H O. Pancreatic function and pancreatic disease studied by means of a secretin. *Acta Med Scand Suppl* 181: 1 1942
- 13 Lowry O H, Rosenbrough N, Farr A L & Randall R J. Protein measurements with the Folin phenol reagent. *J Biol Chem* 193: 265 1951
- 14 Schmid W C, Phillips S F & Samroenkuhl W H J. Jejunal secretion of electrolytes and water in nontropical species. *J Lab Clin Med* 73: 773 1969
- 15 Thomsen R E. Magnesium (fluorimetric). In *Standard methods of chemical analysis*, vol 5. Academic Press, New York and London 1965: p 131
- 16 Thureborn E. Human hepatic bile composition changes due to altered enterohepatic circulation. *Acta Chir Scand Suppl* 303: 1 1962
- 17 Zimmerman M J, Creling D A, Rosenberg R & Janowitz H D. Secretion of calcium by the canine pancreas. *Gastroenterology* 47: 865 1967
- 18 Zoppi G, Hitzig W H, Shmerling D H, Plutz H, Hadorn B & Prader A. Protein content and pancreatic enzyme activities of duodenal juice in normal children and in children with exocrine pancreatic insufficiency. *Helv Paediatr Acta* 23: 577 1968

Submitted Febr 20 1970

Accepted April 20 1970

(G Z) Clinica Pediatrica dell'Università  
Arcispedale S. Anna  
44100 Ferrara  
Italy

**Key words:** Duodenal juice, pancreozymin and secretin stimulation, normal children, cystic fibrosis

## CASE REPORT

### RAPID GROWTH AND CENTROLOBULAR EMPHYSEMA AFTER STENOSING TRACHEITIS AND CONTINUOUS EXPOSURE TO LOW CONCENTRATIONS OF OXYGEN

CHARLES P BIEBER ROBERT C ROSAN WILLIAM H NORTHWAY JR  
PENELOPE CAVE SMITH WILLIAM J DAILY and MICHAEL W BRAND

*From the Departments of Pathology Radiology Anesthesiology and Pediatrics  
Stanford Medical School Palo Alto California USA*

Lobular emphysema of the newborn which was not mentioned in pediatric textbooks prior to the study of Wilton & Milutiy in 1960 (21) now occurs in near-epidemic form (13 18 19). Robertson *et al* were the first to associate the triad of hyaline membrane disease prematurity and oxygen toxicity with cystic emphysema (16). Although it is clear that no one of these factors is essential (6) premature birth (6 19) and low birth weight (6 14) propitiate oxygen toxicity. It was early suggested that the emphysema of oxygen toxicity was largely caused by bronchiolar lesions (11 13 17) an opinion later corroborated by Hawker *et al* (7) and Guillard (6). Oxygen is not unique in this respect viral agents which cause neonatal bronchiolitis may also cause emphysema (1). The unique cellular structure of the neonatal bronchiole may render the premature infant vulnerable to emphysema after bronchiolitis (10).

With these thoughts we present the case of a very small premature infant who was long exposed to low concentrations of supplementary oxygen but who also had retrolental fibroplasia pneumonia, and stenosing tracheitis. He

is the first infant with centrolobular emphysema who died when his cardiopulmonary condition was almost within normal clinical limits.

## CASE REPORT

**Clinical history.** M C was an 846 g male whose gestational age was estimated at 28 weeks and neurological age at 32 weeks. The maternal and family history is not pertinent. The 1 minute Apgar score was 1. Although he had respiratory pauses mild retractions slight cyanosis and infrequent grunting for the first 12 hours his chest radiographs were normal. He ceased grunting after 12 hours. At 1 day he was transferred to our hospital. During the next 8 days he had numerous short bouts of apnea. These were treated with physical stimulation and 100% oxygen delivered briefly by bag and mask. He received a total of about 2100 hours of oxygen at concentrations exceeding 40% during the first 116 days of life as shown in Fig 1.

When he became ill on the 8th day cultures were obtained. These demonstrated *Pseudomonas* in the cerebrospinal fluid conjunctivae and stool. Despite treatment with Kanamycin<sup>®</sup> and Colistin<sup>®</sup> he developed radiographic infiltrates of both lungs and an exacerbation of the apneic episodes. There fore he was intubated in a Bennett positive pressure ventilator for the next 5 1/2 days (20). This therapy included endobronchial suction each hour to remove sticky secretions (12). By the 12th day the right lung field was radiographically opaque and there was a pseudomembranous tracheitis. Cytological examination of his endobronchial secretions during the exposure to oxygen via the ventilator showed an increasingly bizarre atypia a marked diminution of the ciliated

This investigation was supported by USPHS-NIH grant FR #1 and the Council for Tobacco Research—USA grant 644.

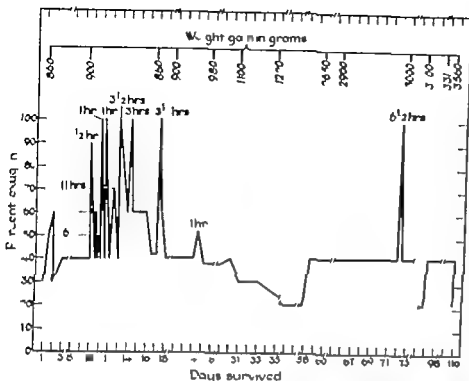


Fig 1 The administration of more than 2100 hours of oxygen at concentrations of 40% or more is shown graphically. There is one period of assisted ventilation beginning on day 10 and extending 5 1/2 consecutive days during which a total of 25 1/2 hours of oxygen was administered at concentrations exceeding 60% via a Bennett machine. The excellent weight gain which begins on day 21 was accompanied by normal growth and development throughout the remainder of the infant's life of 4 1/2 months. The major problem up to day 12 was apnea and extreme prematurity; to day 18 pneumonia; to day 116 and thereafter tracheitis.

cell population and only moderate inflammatory exudate (12).

During the next 2 weeks he followed an accelerating course of progressive radiological, clinical and cytological improvement (12) even though he had tracheitis and recurrent atelectases. When he was 10 weeks old his birth weight had tripled. At this time the second clinical stage of retrolental fibroplasia (15) was initially observed. The records showed that the  $P_{aO_2}$  of aortic blood had exceeded 160 mm Hg only once.

During his 14th week an elective tracheostomy was performed for a stenosis which had enveloped the entire trachea. Despite diverse complications of stomal infection, pneumothorax, pneumomediastinum and dislocation of the prosthesis he thrived. In the 25th week he was sent home with a permanent tracheostomy. He weighed 3950 g.

He was readmitted for study 2 weeks later. His physician noted an alert, well-hydrated infant with somewhat excessive ventilatory motions but without retractions, rales or ronchi. The radiologist found only a few strand-like shadows in the right upper lung field. The urinalysis and hemogram were completely normal. He weighed 4100 g. On the 2nd hospital day he was discovered lifeless in his crib with the endotracheal tube dislodged.

**Autopsy.** Significant lesions were found only in the trachea, lungs, eyes and brain. Both lungs were stiff and knobby. Dilated airspaces were easily seen through the glistening pleurae, and on cut section throughout the lung. The lymphatics were prominent. While the middle lobe occupied two thirds of the right pleural cavity, the right upper lobe was atrophic, consolidated and focally atelectatic. Although collapsed at autopsy, the left lung resembled the right when inflated with fixative but its lobes were in normal



Fig 2 The fixed lung is a faithful replica of the lung *in situ* at autopsy. The many small grey spots are emphysematous subpleural alveolar ducts. On cut section they were irregularly distributed throughout all lobes. The contracted intersegmental markings are most often associated with hyperplastic lymphatics. The lobular bronchioles were dilated and ectatic but there were no mucous plugs. Although the pulmonary arterioles were mildly hyperplastic, the heart was within normal limits for size, weight and thickness of ventricular myocardium.

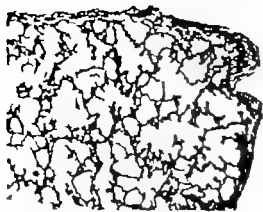


Fig 3 The alveolar ducts are capacious and the alveolar sacs have a simplified appearance because the interalveolar septae are short and blunt. Even partly collapsed airways do not have a normal delicately septate pattern. There is a small degree of interstitial and pleural fibrosis. The irregularity of the pattern and the juxtaposition of small and large alveolar ducts is characteristic.

proportion. The dilated air spaces were histologically identified as distended respiratory ducts associated with ectatic bronchioles and stubby elastin rich alveolar septae. The centriolobular emphysema was accompanied by a feeble interstitial fibrosis involving delicate fibrils rather than cells. There was a muscular hypertrophy of pulmonary arterioles and fragmentation of elastin in large arteries. The trachea though remarkably narrowed showed little acute inflammation. The striking gross lung features are shown (Fig 2) and the histology (Fig 3).

The brain weighed 590 g. There was some abnormal infratentorial firmness and there was correspondingly (3, 4) a marked histological fibrillary gliosis in the optic nerves and chiasm. Perisquamosal grey matter produced longitudinal fasciculus raphe cerebellar peduncles pyramids whole matter of the medulla and the anterior and lateral columns of the spinal cord. In the areas of gliosis there was often an increase in the number of capillaries. No part of the brain demonstrated neuronal loss or necrosis. In the retina there was gliosis of the inner plexiform layer and vasoproliferation in the superotemporal quadrant of the globe.

## DISCUSSION

The autopsy revealed a surprising degree of emphysema which had evaded careful clinical and radiological study and had not prevented rapid growth. This is not Wilson-Milky disease (21) nor pulmonary dysmaturity (2) syndromes which lack the combination of viscid secretions and atypical cytology followed by vascular and

bronchiolar lesions with centriolobular emphysema of this case. That combination does occur in oxygen toxicity (6, 7, 11, 12, 13) and perhaps other forms of neonatal bronchiolitis.

Other causes of airway obstruction in this infant were the pseudomonas infections of lungs and trachea and the tracheal scar. While recognizing the association of *Pseudomonas* with emphysema in cystic fibrosis we find no references to lobular emphysema after bacterial pneumonia of newborns without cystic fibrosis. The possibility of tracheitis is more challenging since most pulmonary resistance is classically in the large airways. What might happen to a rapidly growing lung during severe tracheal obstruction is purely conjectural for there are no studies on the subject. A priori tracheal obstruction does not explain proximal lobular emphysema nor pulmonary vascular change. The possibility of the lesion of the mechanical ventilator must be considered but this is supposed to be interstitial rather than bronchiolar (9). Viral disease is also a possibility (1).

If the disease is due to oxygen intoxication it has occurred at lower concentrations and over a longer course than heretofore considered usual. Nevertheless this possibility remains.

The brain lesions are classic for chronic hypoxia of infancy and are basically a disturbance of white rather than grey matter (3, 4). On the other hand the retrolental fibroplasia was probably related to a very early and short period of hyperoxia (14). Indeed it would be interesting to know if the effects of oxygen on retinal vasculature (14) and pulmonary vasculature (8) were similar.

## SUMMARY

An 846 g newborn survives critical apnea pseudomonas pneumonia and stenosing tracheitis and thrives during 3 months of exposure to 40% oxygen. When he dies unexpectedly during an excellent convalescence his lungs show a surprisingly advanced centriolobular emphysema. The history excites skepticism about the clinical evaluation of silent

lungs of newborns and demonstrates how remarkable a disparity may exist between ventilatory function and morphological damage in newborns. The possibility for contributory etiological roles is discussed for oxygen therapy, tracheal obstruction and rapid growth but not for pneumonia. The case recalls the disturbing increase in neonatal emphysema since 1960. Restricted use of the term Wilson-Mikity disease is urged while generic use of the term lobular emphysema, is condoned.

## REFERENCES

- 1 Adhikari P A, Rao N V & Ferguson C A. Chronic obstructive lung disease following viral bronchopneumonia. *Pediatr Res* 2: 22, 1968.
- 2 Baghdasarian O M, Avery M E & Nubhaus E II D. A form of pulmonary insufficiency in premature infants. Pulmonary dysmaturity? *Am J Roentgenol* 89: 1020, 1963.
- 3 Brand M M. Neuropathological observations on the respiratory distress syndrome of the newborn. In *Drugs and Poisons in Relation to the Developing Nervous System*. USPHS Pub No 1791, 1967, p 192 ff.
- 4 Brand M M & Bignami A. The effects of chronic hypoxia on the neonatal and infantile brain. *Brain* 92: 233, 1969.
- 5 Cleland R. Personal communication to author (R.C.R.) 1966.
- 6 Grillard J L J. Pathologisch anatomische bevindingen bij langlevende berisemde pasgeborenen. In *Boerhaave Christus en voorafgeleid Medisch Onderwijs, Fysiologie en Pathologie in de Perinatale Periode*. Rijksuniversiteit Leiden, Nederl. 1969, pp 34-35.
- 7 Hawker J M, Reynolds E O R & Taghizadeh A. Pulmonary surface tension and pathological changes in infants dying after respirator treatment for severe hyaline membrane disease. *Lancet* II 75, 1967.
- 8 Kristler G S, Caldwell P R B & Weibel. Development of the fine structural damage to alveolar and capillary lining cells in oxygen poisoned rat lungs. *J Cell Biol* 33: 605, 1967.
- 9 Lebow A A, in A A Lebow & D E Smith (eds). *The Lung*. Williams & Wilkins, Baltimore, 1963, p 332 ff.
- 10 Lauweryns J M & Rosan R C. The unit lobule

A revised concept of the neonatal lung. *Abstr 2nd Europ Congr Perinatal Med*. In press.

- 11 Northway W H Jr & Rosan R C. Radiographic features of pulmonary oxygen toxicity in the newborn bronchopulmonary dysplasia. *Radiol* 91: 49, 1968.
- 12 —. Oxygen therapy hazards in the neonate. *Hosp Pract* 4: 59, 1969.
- 13 Northway W H Jr, Rosan R C & Porter D Y. Pulmonary disease following respiratory therapy of hyaline membrane disease. Bronchopulmonary dysplasia. *New Eng J Med* 276: 357, 1967.
- 14 Northway W H Jr, Rosan R C, Shahan L Jr, Castellino R A, Ojeyes M T & Donbridge, T. Radiologic and histologic investigation of pulmonary oxygen toxicity in newborn guinea pigs. *Invest Radiol* 4: 148, 1969.
- 15 Patz A. The role of oxygen in retrolental fibroplasia. *Transact Am Ophthalm Soc* 66: 940, 1963.
- 16 Robertson B, Tunell R & Rudhe U. Late stages of pulmonary hyaline membranes of newborn. *Acta Paediatr Scand* 53: 443, 1964.
- 17 Rosan R C, Porter D Y & Northway W H Jr. Pulmonary dysplasia following survival from severe respiratory distress syndrome (RDS) of newborn new disease. *Fed Proc* 25: 603, 1966.
- 18 Rudhe U & Broberger U. Roentgenological observations in survivors of clinical hyaline membrane disease of the newborn. *Ann Radiol (Paris)* 10: 289, 1967.
- 19 Thibault W W, Grossman H, Hagstrom J W C & Auld P A. Radiological findings in the lungs of premature infants. *J Pediatr* 74: 1, 1969.
- 20 Thomas D V, Fletcher G, Sunshine P, Schafer I A & Klaus M. Prolonged respirator use in pulmonary insufficiency of newborn. *JAMA* 193: 183, 1965.
- 21 Wilson M G & Mikity V G. New form of respiratory disease in premature infants. *Amer J Dis Child* 99: 499, 1960.

Submitted Jan 24, 1970

Accepted March 16, 1970

(Ch P B) Dept of Pathology  
Stanford University Medical School  
Palo Alto  
California 94304  
USA

**Key words:** Neonatal bronchotracheitis, oxygen toxicity, lungs, retrolental fibroplasia, lobular (centrolobular) emphysema.

## CASE REPORT

### HYPERLIPIDEMIA IN TYPE III GLYCOGENOSIS

BOHUMIL VÍTEK, DANUŠE ŠRÁČKOVÁ, MIROSLAV TOMAN, JAROSLAV KRÁTKÝ  
and JIŘÍ VOGNÁREK

From the Second Pediatric Clinic (Head M. Toman), the Clinical Central Laboratory (Head B. Víttek) and the Department of Pathology (Head M. Dlouhý) Hospital for Sick Children Brno Czechoslovakia

Abnormal glycogenolysis in types I, III and VI glycogen storage diseases causes marked changes in the lipid metabolism (7, 9, 11, 15, 27, 28). Insufficient breakdown of hepatic glycogen to glucose leads to frequent hypoglycaemia where the main source of energy becomes free fatty acids that can be metabolized by various tissues, especially the heart and the skeletal muscle. When fat mobilization occurs, free fatty acids are mainly bound to albumin (24).

This report describes a 4-year observation of a boy suffering from type III glycogenosis associated with hyperlipidemia.

## CASE REPORT

A 4-year-old male, the third child of healthy, nonconsanguineous parents, was born after a normal term pregnancy, birth weight 3700 g. When 3 months old, the first symptoms of the disease appeared. There occurred repeated attacks of unconsciousness, especially during upper respiratory infections. At the same time the abdomen became enlarged. The patient was first admitted to our department at the age of 6 months. His weight was 6380 g. Medical examination revealed a physically underdeveloped infant. There was marked muscular hypotonia and the skin was somewhat pale and without efflorescence. The heart was not enlarged; there was no significant protomegasy; no murmur except second heart sound without accentuation. The lung examination was normal. The liver was firm and tender and palpable 4 cm below the costal margin and the spleen was not palpable.

The diagnosis of a glycogen storage disease was made on the basis of biochemical investigations (2, 14). Liver biopsy and later skeletal muscle biopsy. Fasting hypoglycaemia was often accompanied by acetonuria. The serum and blood glucose response to epinephrine and intramuscular glucose was normal after fasting but demonstrable after previous meal (Fig. 1). The glucose was determined by the glucose oxidase method. The glycogen content of the patient's erythrocytes was 3164 µg/g Hb, the parents 124 µg/g Hb and 146 µg/g Hb (23). Liver biopsy showed the following: the hepatocytes were composed of large and pale vacuolated cells. The liver cells contained large amounts of PAS-positive material. The skeletal muscle (in quadriceps) glycogen content was 618 g/100 g of wet tissue (28).

The results of repeated laboratory investigations made on admission and in the 4-year period of observation appear in table. Laboratory studies made on admission revealed elevated alpha-globulin (26), SGOT and SGPT (25). The other measured things were without abnormalities.

The electrocardiogram and X-ray films of the chest revealed no abnormalities.

Examination at the age of four revealed a markedly retarded physical development, his height was 89 cm and weight 14 kg. The skin was rather pale, he had doll-like faces with xanthelasma on the upper eyelids. Hepatomegaly without cardiomegaly and splenomegaly was present, no heart murmur was heard and the second heart sound was slightly accentuated and closely split. There were no abnormalities in the lungs. The X-ray showed no changes either in the heart or the lungs. Blood pressure was 90/60 mm Hg. The ECG was normal with sinus rhythm and AQR5+60 without abnormality. Cardiac catheterization was normal except for a slight elevation of right ventricular pressure (40 mm Hg max, 6 mm Hg min).

After an intramuscular administration of glucose, blood from the vena hepatica and vena cava superior

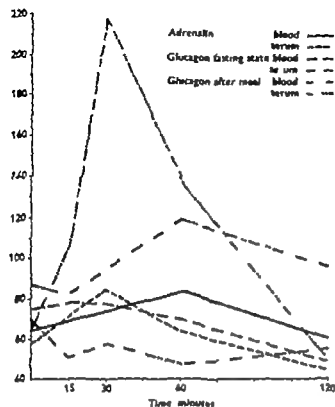


Fig. 1 Response of serum and blood glucose to epinephrine and glucagon

was sampled (Fig. 2). The administration of glucagon did not increase hepatic glycogenolysis whereas the level of free fatty acids (21) in both the vena hepatica and vena cava superior increased. The plasma lactate was found to have remained increased. The plasma pyruvate in the hepatic vein was markedly elevated.

#### Table 1 Biochemical values

Blood lactate and pyruvate was estimated with lactate dehydrogenase using the reagent sets TC-B, TC-C and TC-M (Boehringer Mannheim, Germany). The other methods of analysis for the data in the table were described previously (4, 12, 20, 21, 25, 26).

Age	6 mo	11 mo	13 mo	19 mo	21 mo	4 y
Sedimentation rate	8-12	8-14	—	—	56-70	73-125
Electrophoresis of the serum proteins (in %)						
Albumin	55.0	42.0	50.0	—	—	44.2
alpha <sub>1</sub> glob	5.7	4.2	5.0	—	—	7.6
alpha <sub>2</sub> glob	10.3	15.8	8.0	—	—	15.2
beta glob	10.8	15.8	15.5	—	—	7.6
gamma glob	18.2	22.2	21.5	—	—	25.4
Total lipids (mg/100 ml)	320.0	457.0	899.0	630.0	3 380.0	2 220.0
Lipoproteins (mg/100 ml)	520.0	—	—	1 485.0	—	1 740.0
Total cholesterol (mg/100 ml)	170.0	243.0	175.0	520.0	—	958.0
Serum free fatty acids (μEq/liter)	—	—	—	—	3.6	3.3
Serum bilirubin (mg/100 ml)	0.1	1.5	0.8	0.8	0.9	1.2
Thymol turbidity (units)	5.0	12.9	7.5	3.1	6.1	3.6
SGOT (micromol)	8.84	4.32	3.84	3.0	3.5	3.3
SGPT (micromol)	7.31	4.68	3.0	3.2	3.3	3.0
Lactate (mg/100 ml)	—	—	—	26.0	—	51.0
Pyruvate (mg/100 ml)	—	—	—	1.6	—	1.6

Blood ketone bodies were not measured but there was marked acetonaemia.

The high levels of total lipids (12), lipoproteins (4), cholesterol (12) and the higher percentage of alpha and beta globulins in the serum electrophoresis (26) indicated marked changes in lipid metabolism (Table 1). The blood glycogen concentration had increased to 38.4 mg/100 ml (24). The structure of glycogen isolated from erythrocytes was determined by iodine complex absorption (16). The curve showed deflections from the normal course (A) and had two maximum absorptions (Fig. 3). The first occurred at a wave length of 500 mμ, the second at a wave length of 410 mμ. Glycogen isolated from the erythrocytes of a healthy individual had a maximum iodine absorption at a wave length of 460 mμ. It is not easy to interpret the findings. The maximum absorption of the glycogen at a wave length of 410 mμ could indicate the presence of limit dextrins whereas the presence of the second maximum absorption at a wave length of 500 mμ could rather indicate the presence of amylopectin. Enzyme determination of glycogen was not carried out.

#### DISCUSSION

Recurring hypoglycaemia reflecting defective hepatic glycogenolysis is common to type I, III and VI glycogenoses. Type I glycogenosis is characterized by glucose-6-phosphatase deficiency making release of glucose from glucose-6-phosphate impossible (11). Because of a deficiency of amylase glucosidase in type

III glycogenosis glucose release from glycogen can be effected only from 1,4 glycosidic outer chains. In type VI glycogenosis the deficiency of hepatic phosphorylase also prevents a release of sufficient glucose from the outer chains of hepatic glycogen (6, 14). The deficiency of amylo-1,6-glucosidase in type III can also be demonstrated in the skeletal and heart muscle, the leucocytes and erythrocytes. In type I glycogenosis the deficiency of glucose 6-phosphatase has been shown to occur also in the kidneys and the mucous membrane of the small intestine (11).

The main hormonal regulators of glycogenolysis are glucagon and epinephrine. Glucagon affects only hepatic glycogenolysis (3, 13, 18, 19) and epinephrine affects the glycogenolysis of the muscle and other tissues as well. The effects of glucagon and epinephrine may be due to activation of the phosphorylase system

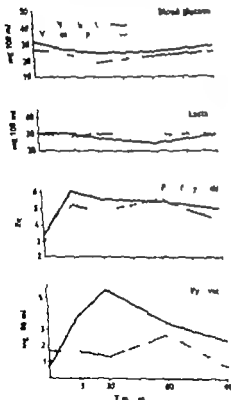


Fig. 7 Results of intramuscular administration of glucagon.

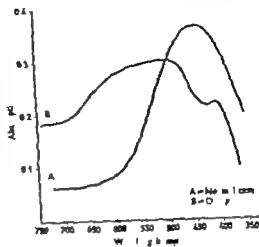


Fig. 3 Absorption spectrum of glycogen iodine complex.

be increasing the production of adenosine 3',5'-cyclic monophosphate through adenyl cyclase activation (3, 29). Glucagon, epinephrine, nor epinephrine, and cyclic AMP were also found to stimulate gluconeogenesis from lactate and pyruvate in the perfused rat liver (8).

In type III glycogenosis during fasting glucose from the 1,4 glycogen linkages is depleted. Further phosphorylase activation by glucagon or epinephrine stimulation cannot therefore cause additional liberation of glucose from phosphorylase limit dextrin; therefore no hyperglycaemic response will be observed. The hyperglycaemic response to glucagon occurs only after a meal because the synthesis of glycogen is normal in type III glycogenosis (13, 18). As the frequency and duration of relative hypoglycaemic intervals increase, free fatty acids and perhaps also glycerol from adipose tissues become the main source of energy (1, 15, 19, 22). Hypoglycaemia may be an important factor in the pathogenesis of hyperlipidemia, hyperlipoproteinemia and hypercholesterolemia.

Elevated free fatty acid levels through enhanced re-esterification of fatty acids could result in an increase of triglyceride synthesis in the liver and subsequent release of lipoproteins into the serum (15). Administration



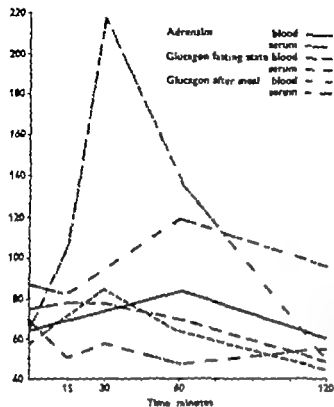


Fig 1 Response of serum and blood glucose to epinephrine and glucagon

was sampled (Fig 2). The administration of glucagon did not increase hepatic glycogenolysis whereas the level of free fatty acids (21) in both the vena hepatica and vena cava superior increased. The plasma lactate was found to have remained increased. The plasma pyruvate in the hepatic vein was markedly elevated.

Table 1 Biochemical values

Blood lactate and pyruvate was estimated with lactate dehydrogenase using the reagent sets TC-B, TC-C and TC-M (Boehringer Mannheim Germany). The other methods of analysis for the data in the table were described previously (4, 12, 20, 21, 25, 26).

Age	6 mo	11 mo	13 mo	19 mo	21 mo	4 y
Sedimentation rate	8-12	8-14	—	—	56-70	73-125
Electrophoresis of the serum proteins (in %)						
Albumin	55.0	42.0	50.0	—	—	44.2
alpha <sub>1</sub> glob	5.7	4.2	5.0	—	—	7.6
alpha <sub>2</sub> glob	10.3	15.8	8.0	—	—	15.2
beta glob	10.8	15.8	15.5	—	—	7.6
gamma glob	18.2	22.2	21.5	—	—	23.4
Total lipids (mg/100 ml)	320.0	457.0	899.0	630.0	3380.0	2220.0
Lipoproteins (mg/100 ml)	520.0	—	—	1485.0	—	1730.0
Total cholesterol (mg/100 ml)	170.0	243.0	275.0	520.0	—	958.0
Serum free fatty acids (mEq/liter)	0.1	1.5	0.8	2.8	3.6	3.3
Serum bilirubin (mg/100 ml)	5.0	12.9	7.5	3.1	6.1	3.6
Thymol turbidity (units)	8.84	4.32	3.84	3.0	3.5	3.3
SGOT (micromol)	7.31	4.68	3.0	3.2	3.3	3.0
SGPT (micromol)	—	—	—	26.0	—	51.0
Lactate (mg/100 ml)	—	—	—	1.6	—	1.6
Pyruvate (mg/100 ml)	—	—	—	—	—	—

Blood ketone bodies were not measured but there was marked acetonaemia.

The high levels of total lipids (12), lipoproteins (4), cholesterol (12) and the higher percentage of alpha and beta globulins in the serum electrophoresis (26) indicated marked changes in lipid metabolism (Table 1). The blood glycogen concentration had increased to 38.4 mg/100 ml (28). The structure of glycogen isolated from erythrocytes was determined by iodine complex absorption (16). The curve showed deflections from the normal course (A) and had two maximum absorptions (Fig 3). The first occurred at a wave length of 500 mμ, the second at a wave length of 410 mμ. Glycogen isolated from the erythrocytes of a healthy individual had a maximum iodine absorption at a wave length of 460 mμ. It is not easy to interpret the findings. The maximum absorption of the glycogen at a wave length of 410 mμ could indicate the presence of limit dextrin whereas the presence of the second maximum absorption at a wave length of 500 mμ could rather indicate the presence of amylopectin. Enzyme determination of glycogen was not carried out.

## DISCUSSION

Recurring hypoglycaemia reflecting defective hepatic glycogenolysis, is common to type I III and VI glycogenoses. Type I glycogenosis is characterized by glucose 6 phosphatase deficiency making release of glucose from glucose 6 phosphate impossible (11). Because of a deficiency of amylo 1,6 glucosidase in type

synthesis of unsaturated fatty acids in blood serum *Physiol Bohemoslov* 10 98 1961

22. Sencer B & Londen L. Functional differentiation of glycogenoses of the liver with respect to the use of glycerol *New Eng J Med* 279 965 1968
23. Selby J B Corbitt M Fisher J & House E. Glycogen in erythrocytes of patients with glycogen storage disease *Pediatrics* 27 103 1961
24. Spector A A. The transport and utilization of free fatty acid *Ann NY Acad Sci* 149 768 1968
25. Sevela M. To the photometric estimation of serum transaminase: comparison of Warman-Frankel method and the electrophoretic method *Vnitřní lek (Praha)* 4 721 1958
26. Tuma A. Die Elektrophorese der Blutsäurenproteine an den Zelluloseacetatschichten *Jahrbuch Sci Univ (Brno)* 31 134 1966
27. Vitek B. Hemiplegie u dvou dětí s glykogenózou a fibrilací endokardu *Cs Pediatr* 24 503 1969
28. Vitek B Kracková H Rozpurnová L & Šnopyková J. Glykogenóza a její diagnostika *Cs Pediatr* 24 567 1969
29. Wherry A J Shum A Trubow M & Fox P P. Effect of glucagon and insulin on serum fatty acids in normal and depancreatized dogs *Proc Soc Exp Biol Med* 130 35 1969

Submitted Dec 2 1969

Accepted April 6 1970

(B V) 2nd Pediatric Clinic  
Brno, Czechoslovakia

Key words: Hyperlipidemia, glycogenosis type III

of glucagon effected an increase of free fatty acids in our patient 12 hours postprandial, but did not produce a hyperglycaemic response. This can be explained by the increased mobilization of fat from adipose tissues in the liver (29) and perhaps even from peripheral fat by tissue lipase stimulation as has been demonstrated *in vitro* (5).

The influence of glucagon on fat mobilization is difficult to define *in vivo* in most instances since the decrease in free fatty acids observed may be a consequence of the concomitant hyperglycaemia *per se* (17). An elevation of pyruvate in the hepatic vein without an elevation of lactate after glucagon should be very likely originate from glycerol which after hydrolysis changes to inose compounds (Di hydroxyacetone and glyceraldehyde) and joins the main metabolic pathway of carbohydrate (22, 24).

## SUMMARY

During 4 years of observation, a patient suffering from type III glycogenosis developed considerable hyperlipidemia with hyperlipoproteinemia and hypercholesterolemia. The boy repeatedly suffered from hypoglycaemia especially during infections. Our diagnosis was made on the basis of markedly increased quantities of glycogen in the liver, skeletal muscle and the erythrocytes. After a 12-hour fast, glucagon administration resulted in an elevation of free fatty acids and pyruvate, no change in lactate and no increase in glucose as measured in both the hepatic and peripheral veins. These observations can be explained by the influence of glucagon on lipolysis.

## REFERENCES

- Bolinger R E, Shase S R & Kirkpatrick Ch C. Secondary rise in plasma free fatty acids following glucose load. *J Clin Endocrin Metabol* 22 873 1962.
- Breton A, Guider B, Ponté C & Lescut J. Polycose glycoénuque. Contribution des tests cliniques au diagnostic de la forme enzymatique. *Pédiatrie (Lyon)* 15 635 1960.
- Buchanan K D, Vance J E, Dunst J, Williams R H, Hubermell B & Pace S. Effect of blood glucose on glucagon in anesthetized dogs. *Diabetes* 18 11 1969.
- Burstein M & Samaille J. Dosage des b- $\alpha$  lipoprotéines sériques après précipitation sélective par l'heparine. *Presse Med* 66 974 1958.
- Camu F. Interactions du glucagon de l'insuline et du glucose sur la lipolyse du tissu adipeux de rats *in vitro*. *Arch Int Pharmacodyn* 178 370 1968.
- Cori G T & Larner J. Action of amylase-16-glucosidase and phosphorylase on glycogen and amylopectin. *J Biol Chem* 188 17 1951.
- Crevelde S Van & Huijng F. Glycogen storage disease. Biochemical and clinical data in sixteen cases. *Amer J Med* 38 554 1965.
- Exton J H & Park C R. Control of gluconeogenesis in liver. III Effects of lactate, pyruvate, fructose, glucagon, epinephrine and adenosine 3',5' monophosphate on gluconeogenic intermediates in the perfused rat liver. *J Biol Chem* 244 1424 1969.
- Fernandes J & Pikaar N A. Hyperlipemia in children with liver glycogen disease. *Amer J Clin Nutr* 22 617 1969.
- Forbes G B. Glycogen storage disease. Report of a case with abnormal glycogen structure in liver. *J Pediatr* 42 645 1953.
- Fuhrmann W, Jakovc S & Haa D Y Y. Glycogenose Typ I (v. Gierke) trüber mitgeteilt als familiäre Hyperlipidemie. *Dtsch Med Wochr* 91 453 1966.
- Homolla J. *Klinické biochemické diagnostické metody*. Stát zdrav. nákl. Praha 1969.
- Hug G. Glycogen tolerance test in glycogen storage disease. *J Pediatr* 60 545 1962.
- Hug G, Garancis J C, Schubert W K & Kaplan S. Glycogen storage disease types II, III, VIII and IX. A biochemical and electron microscopic analysis. *Amer J Dis Child* 111 457 1966.
- Jakovc S, Krichadonian A K & Haa D. The hyperlipidemia in glycogen storage disease. *J Clin Lab Med* 68 769 1966.
- Kruman C R. A method for the colorimetric estimation of glycogen with iodine. *Anal Biochem* 4 17 1962.
- Lefebvre P. Glucagon et taux sanguin des acides gras non estérifiés chez l'homme. *Ann Endocrin* 26 602 1965.
- Limbeck G A & Kelley V C. Double barrel glucagon test. *Amer J Dis Child* 109 162 1965.
- Lowe C U, Sokal J E, Mosovich I I, Sarcone E J & Doray H H. Studies in liver glycogen disease. Effects of glucagon and other agents on metabolic pattern and clinical status. *Amer J Med* 33 4 1962.
- MacLogan N F. Liver function tests in the diagnosis of jaundice. *Anal Chem* 25 815 1953.
- Novák M. An ultramicromethod for the deter-

- metabolism of unsaturated fatty acids in blood serum *Physiol Bohemoslov* 10: 98 1961
22. Sencer H & Londea L. Functional differentiation of glycogenoses of the liver with respect to the use of glyceral *New Eng J Med* 279: 965 1968
  23. Sedbury J B, Cornblith M, Fisher J & House E. Glycogen in erythrocytes of patients with glycogen storage disease *Pediatrics* 27: 103 1961
  24. Spicer A A. The transport and utilization of free fatty acid *Ann NY Acad Sci* 149: 768 1968
  25. Jevla M. To the photometric estimation of serum transaminase: comparison of Reitman-Frankel method and the electrophoretic method *Laborat let (Praha)* 4: 721 1958
  26. Tuma A. Die Elektrophorese der Blutserumproteine an den Zelluloseacetatschichten *Publ fac Sci Univ (Brno)* 31: 136 1966
  27. Vitek B. Hemiplegie u dvou dětí s glykogenózou a fibroblastózou endokardu *Cs Pediatr* 24: 503 1969
  28. Vitek B, Šrámková, D, Rozpurnová L & Šoopková, J. Glykogenóza a její diagnostika *Cs Pediatr* 24: 567 1969
  29. Whitty A J, Shum A, Trubow M & Ford P P. Effect of glucagon and insulin on serum fatty acids in normal and depancreatized dogs *Proc Soc Exp Biol Med* 130: 55 1969
- Submitted Dec 2 1969  
Accepted April 6 1970
- (B V) 2nd Pediatric Clinic  
Brno, Černá Pole  
Czechoslovakia
- Key words:** Hyperlipidemia, glycogenosis type III

## CASE REPORT

### A CASE OF THE XXXXY CHROMOSOME ANOMALY WITH FOUR MATERNAL X CHROMOSOMES AND DIABETIC GLUCOSE TOLERANCE

M FJORD CHRISTENSEN and A J THERMELSEN

*From the University Clinic of Paediatrics Aarhus Kommunehospital and the Institute of Medical Microbiology Aarhus University, Denmark*

The first case of the chromosome anomaly 49,XXXXY was reported in 1960 by Francaro et al (3). In 1966 Zaleski et al (20) reviewed a total of 33 cases. Since then at least nine further cases have been reported (1, 2, 4, 5, 6, 7, 10, 13, 19). Out of these 42 cases 7 were mosaics.

Although the clinical picture has some of the features of the syndromes of Down and Klinefelter it forms a separate entity (20).

A new case of the syndrome is described below in a 2 1/2 year-old boy with a diabetic glucose tolerance test. The case is noteworthy too, because it is the fourth case in the literature where the  $\lambda_g$  bloodtype investigation in the patient and his parents gives information as to the development of the anomaly.

## CASE REPORT

The patient was born as the second of twins on July 18, 1966. The other twin, a girl, was stillborn and macerated. The parents were unrelated. Both were 39 years old at the time of the delivery. The father and a 5 year-old sister were healthy and so was the mother before the pregnancy. Because of nausea she received Torcan® (thethylperazine maleate) 65 mg perorally 3 times a day during the first 2 months of pregnancy. The delivery was medically induced 2 weeks before term because of the following signs in the mother: itching, slight arterial hypertension, proteinuria, oedema, slight jaundice and bilirubinuria. The delivery was uncomplicated. His birth

weight was 1900 g and his crown-heel length 46 cm. Immediately after birth he was very hypotonic with insufficient respiration but normal heart action. He recovered after suction and oxygen supply. At this time the neonatal period was without problems.

He was admitted to the University Clinic of Paediatrics Aarhus on the 27th of January 1969 because of retarded mental and physical development with suspicion of Down's syndrome.

On admission he was 81 cm tall which is 11% below the average height for his age (Fig. 1). His weight was 11.15 kg. The skull was brachycephalic and asymmetrical with flattening of the right occipital region. The ears were low set and slightly malformed. There were slight hypertelorism and epicanthus, a distinct mongoloid slanting of the palpebral fissures. The bridge of the nose was broad and flat. The face was also characterized by a moderate degree of pinnae. The neck was short with loose and abundant skin. The limbs were hypotonic with poorly developed musculature. There were bilateral cubitus valgus and genu valgum and the knee joints could be overextended by about 10 degrees. There was a fourth finger crease but the fifth finger was short and radially incurved on both sides. The scrotum was hypoplastic and the testes could not be palpated. The penis was normal.

There were no signs of organic heart disease. Hearing and sight were judged to be normal. At ophthalmoscopic examination the papillae were slightly flattened and pale.

He was considerably physically retarded. He was able to sit on his own but needed support to stand and even when strongly supported he was only able to make awkward walking movements. The IQ determined by Cattell's infant intelligence scale was 35.

**Radiological examination.** The skull was found acrocephalic and brachycephalic. The extremities showed delayed bone development especially peripherally (bone age 6 months). Second phalanx of the fifth finger



Fig 1 The patient 31 months old.

and the distal phalanx of the first toe were remarkably short. The dorsal vertebrae were deformed and of reduced height and there was spina bifida of the sixth lumbar vertebra. No radio-ulnar synostosis was found.

**Laboratory investigations** The electroencephalogram during sleep was normal. The serum levels of

Table 1 Blood sugar and plasma insulin before and after oral administration of 25 g glucose

Hours	Blood sugar (mg/100 ml)	Plasma insulin (microU/ml)
0	30	7
1/4	57	7
1/2	74	11
3/4	107	15
1	141	28
1 1/2	199	60
2	239	77
1 1/2	245	40
3	154	40

Table 2 Frequency of 3, 2, 1 and 0 Barr bodies in cells from oral mucosa and cells from cultures of skin fibroblasts

No of Barr bodies	3 (%)	2 (%)	1 (%)	0 (%)
Oral mucosa				
1 count	7	29	39	25
2 count	7	31	29	32
Skin				
1 count	27	28	31	14
2 count	30	34	26	10

calcium and thyroxine were normal and so were the microreactions for toxoplasmosis, cytomegalovirus infection and syphilis. The excretion of acid mucopolysaccharides in the urine was within normal limits. In a 24 hours sample of urine there was an excretion of 45  $\mu$ Mol carnitine but apart from this the quantitative determination of the amino acids in the urine and plasma was normal (the determination was carried out on a Technicon Amino Acid Analyzer by the Central Laboratory County Hospital Aalborg). There was no glucosuria. Blood glucose during fast mg was normal but after oral administration of 25 g glucose a diabetic glucose tolerance was found with a slow and not very great rise in plasma insulin (Table 1) (for method see Nielsen et al (14)).

**Cytogenetical investigations** The sex chromosomes were investigated in preparations of oral mucosa stained by the Feulgen method and in cultures of skin fibroblasts grown for 4 days in Leighton tubes and stained with Feulgen light green.

In the preparations from oral mucosa only Barr bodies located at the margin of the nucleus were counted whereas in the fibroblast cultures Barr bodies with juxtanuclear and intermediate location were counted as well. 100 nuclei were counted in each of two preparations from each of the two tissues. The results are seen in Table 2.

Counting and analysis of chromosomes were done in 30 metaphases from cultures of peripheral blood by a modification of the method of Moorhead et al (12) and in 30 metaphases from cultures of a skin biopsy specimen (18) taken from the inside of the upper arm. The distribution of chromosome numbers appears from Table 3. The 3 cells with 48 chromo-

Table 3 Distribution of chromosome numbers

	Chromosome count			
	<47	47	48	49
Peripheral blood	0	0	1	29
Skin	0	1	2	27

some showed E, E and C monosomy and the one cell with 47 chromosomes was 47,XXX,Y with monosomy D.

The chromosomes of both parents were investigated in cultures from peripheral blood and both had normal karyotypes.

**Dermatoglyphic analysis.** Finger and palms: the total ridge count was low: 96 (normal average in men about 145). Triradius distance a-b was 50 / 50\*, b-c 188 / 133 and c-d 31 / 37\*, the left hand being mentioned first (mean values a-b 39, b-c 24, c-d 37\*). The triradius  $\tau$  was displaced to  $\tau'$ . Toes and soles: the pattern intensity was low. First interdigital interval was large.

**Ag blood type.** The father had the type Xg (a+) the patient and his mother Xg (a-).

## DISCUSSION

With the increasing use of chromosome investigations it has been shown among other things that different abnormal karyotypes often have clinical symptoms in common. Our patient was admitted because of suspicion of Down's syndrome but he appeared to be a typical representative of the XXXXY anomaly.

The confusion with Down's syndrome is excusable as the patients with 49,XXXXY are considerably mentally retarded and marked by hypotonia and joint hyperflexibility. As a rule they also show mongoloid slanting of the palpebral fissures, epicanthus, four-finger crease, and short incurved fifth finger. Other features do to some extent separate these patients from patients with Down's syndrome: hypertelorism, prognathism and large malformed ears. Other frequent findings are coxa valga, genu valgum and proximal radio-ulnar synostosis. Finally the patients show features that may call attention to Klinefelter's syndrome: hypogonadism with underdeveloped scrotum, undescended testes and small penis. The common finding in testicular biopsy is atrophy of tubuli seminiiferi. As adults the patients are characterized by eunuchoid proportions and may have gynecomastia.

Often they are born small for dates. Their expectation of life is not known. The oldest one of the reported cases was 35 years at the time of the investigation (11).

It has been discussed whether the mother's age at the time of the patient's birth is higher than normal. This age is stated in 36 cases, the average being 29½ years. This figure should be looked upon with reservation however, as probably some of the patients with the XXXXY syndrome born to older mothers are classified as cases of Down's syndrome without chromosome investigation.

The chromosome anomaly may be caused by meiotic non-disjunction in either egg and/or sperm and eventually by mitotic non-disjunction of the X chromosome in the first divisions of the zygote. Investigation of the Ag blood type sometimes makes it possible to decide whether the four X chromosomes in these patients are maternal or paternal. In 25 mother-father-XXXXY combinations so far investigated (17) only 3 have been informative (11, 13) and in all 3 cases the distribution was as in our case. The patient and his mother were Xg (a-) and the father Xg (a+). Since the Xg gene is dominant all the patient's X chromosomes were maternal and the karyotype therefore 49,X<sup>M</sup>X<sup>M</sup>X<sup>M</sup>X<sup>M</sup>Y. Therefore, the most likely explanation of the development of the anomaly is successive non-disjunction in the meiotic divisions of the egg (16).

Although glucose tolerance tests have been performed in patients with the XXXXY syndrome (8, 9, 20) diabetic glucose tolerance has not been reported before in these patients. In a way this is surprising considering the increased frequency of diabetes of the maturity-onset type in patients with Klinefelter's syndrome (14). The insulin response during peroral glucose tolerance test in these patients is as a rule brisk with the highest values not higher than those found in non-diabetics but longer lasting. In our patient the response is more like that found in patients with mild juvenile diabetes (14). The explanation of the increased frequency of diabetes in Klinefelter's syndrome is under discussion. It is worth noting that a high frequency of diabetes has been found in Turner's syndrome too (15). The genetic unbalance caused by a supernumerary

as well as by a missing X chromosome there fore seems to predispose to diabetes

An increased frequency of diabetes in the mothers of patients with Klinefelter's syndrome has been claimed (14) and some authors have also found an increased frequency of diabetes in the parents of patients with Turner's syndrome (15) If these results are confirmed the most likely explanation is that diabetes mellitus facilitates non-disjunction. There were no known cases of diabetes in our patient's family.

Further investigations are indicated to elucidate these problems however and the investigations should also be extended to cases of the XXXY syndrome to see if there is the same relation between this syndrome and diabetes as between Klinefelter's syndrome and diabetes.

### SUMMARY

The 49 XXXXY anomaly was found in a 2½ year-old boy with signs typical of that anomaly: physical and mental retardation by polydactylism and certain mongoloid features.

Determination of the Xg blood type in the patient and his parents showed that the Y chromosomes were of maternal origin. The most likely explanation of the development of the anomaly is non-disjunction twice during the meiotic divisions of the egg.

The finding of a diabetic glucose tolerance test is discussed in relation to the increased frequency of diabetes mellitus in patients with Klinefelter's syndrome.

### ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Drs R. R. Race and Ruth Sanger London for the analysis of the Xg blood type, Dr G. Schmidt Den Helder for the Xg blood type, Dr J. Lohman Medical Department M. Aarhøus Hospital for the plasma protein determination.

### REFERENCES

1. Flouhy E, Dehmet M & Radauer M Non effe observations du syndrome XXXXY *Ann Pediatr* 44 479 1968

2. Ferrante E, Brua L, Vignetti F & Valeri O M. Sindrome Klinefelter con cariotipo XXXXY in un bambino di 3 anni *Arch Ital Pediatr* 34 275 1966
3. Fraccaro M, Kasper B. & Lindsten J. A child with 49 chromosomes *Lancet* II 899 1960
4. Freilund A. *Klinefelter's Syndrome* Thesis Copenhagen 1969
5. Galmus J & Barr H S. The XXXXY sex chromosome abnormality *Arch Dis Child* 41 12 1966
6. Oedhög A O. Oak nadir bir Klinefelter sen droms XXXXY kromosom konstansu, *Ann Tip Fak Med (Istanbul)* 30 236 1967
7. Grouchy J, de Frelz J, Brian A & Lamy M. Un cas de mosaïque XXXXY/XXXY *Ann Genet* 8 41 1965
8. Jancar J. Mentally defective males with XXXXY chromosomes in J. Oster & H. V. Sier (eds) *Paper presented at international congress on the scientific study of mental retardation Copenhagen 1965* p 179
9. Lamy M, Asselineau M, Grouchy J, de Frelz J, Monique XXXXY/XXXY dans le syndrome de Klinefelter *Ann Pediatr* 39 167 1965
10. Lawns J, Freilund A & Johnsen S O. Sexual differentiation in the XXXXY chromosome constitution *Acta Path Microbiol Scand* 65 321 1965
11. Lawns F J W, Freilund A, Sanger R & Race R R. Some of the X chromosomes in two XXXXY males *Lancet* II 589 1964
12. Moorhead P S, Nowell P C, Mellman W J, Batigs D M & Hungerford D A. Chromosome preparations of leucocytes cultured from human peripheral blood *Exp Cell Res* 20 613 1960
13. Marlen J D & Scholz W. Biologische Untersuchung der Herkunft der überzahligen X-Chromosomen beim XXXXY Syndrom *Blut* 16 164 1967
14. Nielsen J, Johnsen K. & Yde H. Frequency of diabetes mellitus in patients with Klinefelter's syndrome of different chromosome constitutions and the XYY syndrome. Plasma insulin and growth hormone level after a glucose load *J Clin Endocr* 9 1062 1969
15. — The frequency of diabetes mellitus in patients with Turner's syndrome and pure gonadal dysgenesis *Acta Endocr* 62 231 1969
16. Race R R & Sanger Ruth. Xg and sex-chromosome abnormalities *Brit Med Bull* 25 99 1969
17. Sanger R. Personal communication
18. Theilacker A J. Sandwich technique for the establishment of cultures of human skin for chromosome investigation *Acta Path Microbiol Scand* 61 317 1964



somes showed E, E and C monosomy and the one cell with 47 chromosomes was 47,XXXXY with monosomy D.

The chromosomes of both parents were investigated in cultures from peripheral blood and both had normal karyotypes.

*Dermatoglyphic analysis.* Finger and palm: the total ridge count was low 96 (normal average in men about 145). Triradius distance *a-b* was 50% / 50% *b-c* 18.8% / 13.3% and *c-d* 31% / 37% the left hand being mentioned first (mean values *a-b* 39% *b-c* 24% *c-d* 37%). The triradius *t* was displaced to *t* Toes and soles the pattern intensity was low. First interdigital interval was large.

*Blood type.* The father had the type Xg (a+) the patient and his mother Xg (a-).

## DISCUSSION

With the increasing use of chromosome investigations it has been shown among other things that different abnormal karyotypes often have clinical symptoms in common. Our patient was admitted because of suspicion of Down's syndrome but he appeared to be a typical representative of the XXXXY anomaly.

The confusion with Down's syndrome is excusable as the patients with 49,XXXXY are considerably mentally retarded and marked by hypotonia and joint hyperflexibility. As a rule they also show mongoloid slanting of the palpebral fissures, epicanthus, four finger crease and short incurved fifth finger. Other features do to some extent separate these patients from patients with Down's syndrome: hypertelorism, prognathism and large malformed ears. Other frequent findings are coxa valga, genu valgum and proximal radio-ulnar synostosis. Finally the patients show features that may call attention to Klinefelter's syndrome: hypogonadism with underdeveloped scrotum, undescended testes and small penis. The common finding in testicular biopsy is atrophy of tubuli seminiferi. As adults the patients are characterized by eunuchoid proportions and may have gynecomastia.

Often they are born small for dates. Their expectation of life is not known. The oldest one of the reported cases was 35 years at the time of the investigation (11).

It has been discussed whether the mother's age at the time of the patient's birth is higher than normal. This age is stated in 36 cases, the average being 29½ years. This figure should be looked upon with reservation however, as probably some of the patients with the XXXXY syndrome born to older mothers are classified as cases of Down's syndrome without chromosome investigation.

The chromosome anomaly may be caused by meiotic non-disjunction in either egg and/or sperm and eventually by mitotic non-disjunction of the Y chromosome in the first divisions of the zygote. Investigation of the Xg blood type sometimes makes it possible to decide whether the four X chromosomes in these patients are maternal or paternal. In 25 mother-father XXXXY combinations so far investigated (17) only 3 have been informative (11, 13) and in all 3 cases the distribution was as in our case. The patient and his mother were Xg (a-) and the father Xg (a+). Since the Xg gene is dominant all the patient's X chromosomes were maternal and the karyotype therefore 49,X<sup>m</sup>X<sup>m</sup>X<sup>m</sup>X<sup>m</sup>Y. Therefore, the most likely explanation of the development of the anomaly is successive non-disjunction in the meiotic divisions of the egg (16).

Although glucose tolerance tests have been performed in patients with the XXXXY syndrome (8, 9, 20) diabetic glucose tolerance has not been reported before in these patients. In a way this is surprising, considering the increased frequency of diabetes of the maturity-onset type in patients with Klinefelter's syndrome (14). The insulin response during peroral glucose tolerance test in these patients is as a rule brisk with the highest values not higher than those found in non-diabetics but longer lasting. In our patient the response is more like that found in patients with mild juvenile diabetes (14). The explanation of the increased frequency of diabetes in Klinefelter's syndrome is under discussion. It is worth noting that a high frequency of diabetes has been found in Turner's syndrome too (15). The genetic unbalance caused by a supernumerary

## CASE REPORT

### PROLONGED SURVIVAL IN NEUROBLASTOMA WITH MULTIPLE SKELETAL METASTASES AND BONE MARROW INFILTRATION

KATHRYN E. MCGOLDRICK and P. LANZKOWSKY

From the Department of Pediatrics New York Hospital-Cornell University Medical College New York USA

Review of the literature has revealed only two recorded children with disseminated skeletal metastases and bone marrow infiltration secondary to neuroblastoma who have survived for longer than 5 years. In view of the paucity of reported cases of neuroblastoma with osseous and bone marrow metastases who have had long term survival we report an 8 year 2 month old female who is well without evidence of disease 7 years after having had neuroblastoma of the left adrenal gland accompanied by radiographic evidence of multiple bone metastases and histologic evidence of bone marrow infiltration by neuroblastoma.

## CASE REPORT

In October 1961 a 14 month-old Caucasian girl the product of a full term normal gestation and delivery was admitted to New York Hospital and on physical examination an upper quadrant abdominal mass was palpated. Intravenous pyelogram showed a suprapubic mass containing calcium and displacing the left upper collecting system downward. Urinary vanillylmandelic acid excretion was 31.3 mg/24 hours (normal adult range 0.1-9.0 mg/24 hours). Radiographic skeletal survey revealed an irregular lytic defect in the distal metaphysis of the left tibia with periosteal reaction, irregularity of the frontal bone and lysis between the middle wings both femoral heads and right fifth rib (Figs 1 and 2). Biopsy of the left tibia disclosed infiltration of the bone

marrow by nests of darkly staining tumor cells arranged in small nodules.

The patient was treated with irradiation (2,000 r and 1,500 r respectively) to the primary tumor of the left adrenal gland and to the left tibia followed by cyclophosphamide and methotrexate. Cyclophosphamide was administered in a dose of 15 mg per kg body weight intravenously followed by 50 mg per kg daily orally for 2 months and for a further month in a dose of 30 mg per kg on alternate days. Methotrexate was administered orally in a dose of 1.25 mg daily for 2 months and in the same dosage on alternate days for a period of 21 months. She had frequent radiographic skeletal surveys and in May 1964 for the first time she displayed no roentgenologic evidence of metastatic disease. In October 1964 24 hour urinary norepinephrine level was 24.38 µg, epinephrine 5.63 µg and vanillylmandelic acid 113 µg. All three values are within normal limits. In December 1964 the primary tumor and entire left adrenal gland were removed and the left metastatic and para-aortic nodes resected. The resected tissue did not reveal any tumor cells.

In May 1967 the patient presented with signs of small bowel obstruction. Exploratory laparotomy was carried out and adhesions obstructing the small bowel were resected. No abdominal tumor was present at that time and repeat vanillylmandelic acid excretion and roentgenological skeletal survey were normal. The girl was last seen in the New York Hospital Pediatric Hematology Clinic in April 1970 and she continues to be well without any evidence of neuroblastoma clinically, chemically or radiographically.

## DISCUSSION

Neuroblastoma is a disease with a variable clinical course ranging from prolonged remis-

This work was supported by the Children's Blood Foundation Inc.

- 19 Tsenghi C, Metivostou A, Economou Mavrou A & Matsaniotis N. An  $\lambda\lambda\lambda\text{XXY}$  male. *Delt Paidi Klin Ponep Athin* 13 487 1966
- 20 Zaleski W A, Houston C S, Parsons J & Yung K L. The  $\lambda\lambda\lambda\text{XXY}$  chromosome anomaly: report of three new cases and review of 30 cases from the literature. *Canad Med Ass J* 94 1143 1966

Submitted March 2 1970

Accepted April 20 1970

(M F Chr)

University Clinic of Paediatrics

Kommunebo-petitet

Aarhus C

Denmark

**Key words:**  $\text{XX}\lambda\lambda\text{YY}$  chromosome anomaly, diabetes, glucose tolerance, Xg blood type

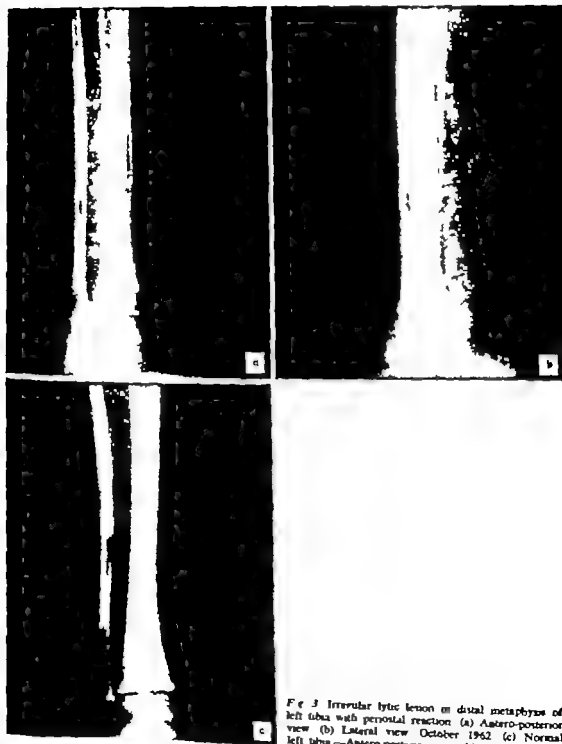


Fig 3 Irregular lytic lesion in distal metaphysis of left tibia with periosteal reaction (a) Antero-posterior view (b) Lateral view October 1962 (c) Normal left tibia—Antero-posterior view May 1964

tion to rapidly disseminating disease and death. Various factors have been considered useful in determining prognosis in this disease. These

factors include age of patient at time of diagnosis, extent of disease, tumor cytology, site of primary and therapy employed (2-4). An



Fig 1 Irregular lytic lesion of frontal bone



Fig 2 Lytic lesions of both orbital wings and femoral necks

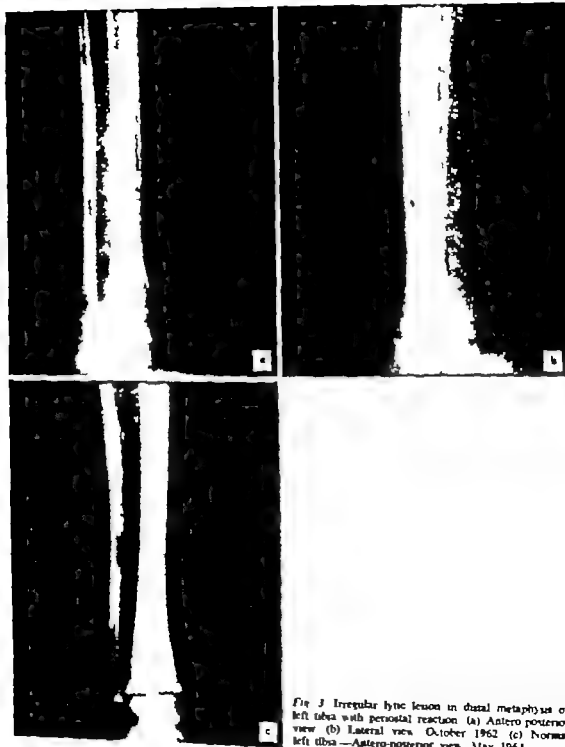


Fig 3 Irregular lytic lesion in distal metaphysis of left tibia with periosteal reaction (a) Antero-posterior view (b) Lateral view, October 1962 (c) Normal left tibia—Antero-posterior view, May 1964

won to rapidly disseminating disease and death. Various factors have been considered useful in determining prognosis in this disease. These

factors include age of patient at time of diagnosis, extent of disease, tumor cytology, site of primary and therapy employed (2, 4). An



Fig 1 Irregular lytic lesion of frontal bone



Fig 2 Lytic lesions of both ethmoid wings and femoral necks

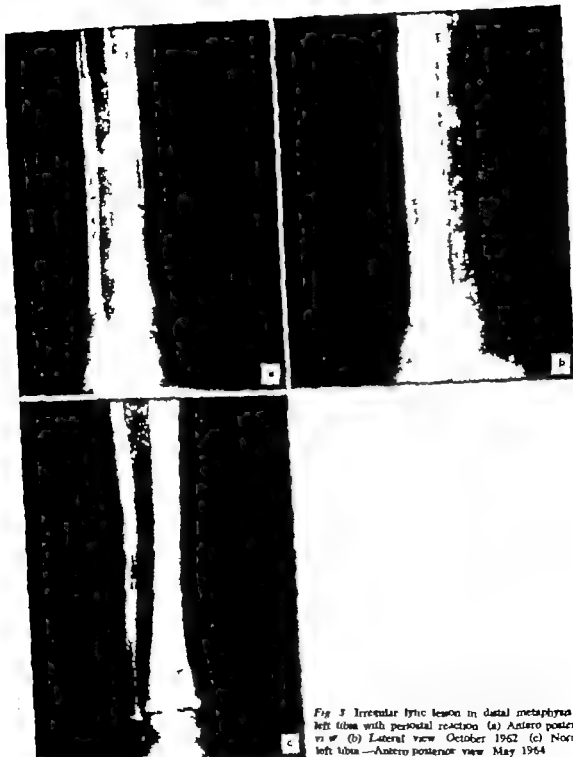


Fig 3 Irregular lytic lesion in distal metaphysis of left tibia with periosteal reaction (a) Antero posterior view (b) Lateral view October 1962 (c) Normal left tibia—Antero posterior view May 1964

sion to rapidly disseminating disease and death. Various factors have been considered useful in determining prognosis in this disease. These

factors include age of patient at time of diagnosis, extent of disease, tumor cytology, site of primary and therapy employed (2, 4). An



especially poor prognosis has been associated with skeletal metastases and/or bone marrow infiltration

With reference to skeletal metastases, Fortner et al (2) reported survival for longer than 5 years in four patients with bony metastases in neuroblastoma in a series of 133 patients but none of their patients with bone marrow infiltration survived for 5 years. Reilly et al (4) reported long term survival of three children with disseminated skeletal metastases secondary to neuroblastoma and in review of the literature they found only eight additional patients who had survived longer than 2 years after the development of metastatic bone lesions.

Bodian (1) and James et al (3) have reported prolonged survival following bone marrow infiltration in neuroblastoma in which there has been no demonstrable roentgenologic evidence of skeletal dissemination. Bodian (1) reported a male infant who presented at 4 months of age with several subcutaneous nodules histologically characteristic of neuroblastoma and with tumor cells present in the bone marrow but no radiologic evidence of osseous dissemination demonstrable. The patient was seen 4 years and 7 months following diagnosis and physical examination, bone marrow examination and urinary vanillyl mandelic acid were normal. James et al (3) reported a 5 week old female infant who presented with metastatic neuroblastoma to bone marrow and lymph nodes. The site of primary tumor remained undetermined as she was given a 12 month course of vincristine and cyclophosphamide with complete remission for 2 years.

There are only two patients on record who have had both skeletal metastases and bone marrow infiltration in neuroblastoma who have

had prolonged survival and both these cases shared the relatively favorable prognostic signs of an extra adrenal primary neoplasm and clinical onset of disease during the first year of life (4). The patient reported in this paper had a tumor which was adrenal in origin and at the time of diagnosis was 14 months of age.

## SUMMARY

The present report is the third recorded case, to our knowledge, of a child with multiple skeletal metastases and bone marrow infiltration secondary to disseminated neuroblastoma who has survived 7 years and 6 months after the diagnosis without clinical, chemical or radiographic evidence of disease.

## REFERENCES

- 1 Bodian M. Neuroblastoma: An evaluation of its natural history and the effects of therapy with particular reference to treatment by massive doses of vitamin B<sub>12</sub>. *Arch Dis Child* 38: 606, 1963.
- 2 Fortner J, Nicastri A, Murphy M L. Neuroblastoma: Natural history and results of treatment in 133 cases. *Ann Surg* 167: 132, 1968.
- 3 James D H, Hustu O, Wrenn E C & Pikel D. Combination chemotherapy of childhood neuroblastoma. *JAMA* 194: 123, 1965.
- 4 Reilly D, Nesbit M E & Krivit W. Cure of three patients who had skeletal metastases in disseminated neuroblastoma. *Pediatrics* 41: 47, 1968.

Submitted Febr. 10, 1970

Accepted May 15, 1970

(P. L.) Department of Pediatrics  
Long Island Jewish Medical Center  
New Hyde Park  
New York 11040 USA

Key words: Neuroblastoma, skeletal metastases, bone marrow infiltration.

## CASE REPORT

# FAMILIAL EARLY HYPOPARATHYROIDISM ASSOCIATED WITH HYPOMAGNESAEMIA

E. NILASSON

From the Department of Paediatrics Central Hospital Uppsala Sweden

Idiopathic hypoparathyroidism depends on a deficiency of parathyroid hormone and is considered to be a relatively rare disease. Patients suffering from idiopathic hypoparathyroidism must fulfil the following criteria: 1) low serum calcium level 2) high serum inorganic phosphorus level 3) absence of radiological signs of rickets and osteomalacia 4) chronic tetany 5) absence of renal insufficiency stomathea chronic diarrhea and alkalosis (8). They should present acute phosphate diuresis following the intravenous administration of parathyroid hormone (29).

It is the purpose of this paper to present two cases of idiopathic hypoparathyroidism occurring in siblings diagnosed during their first year of life with evidence of non sex linked heredity and a low serum magnesium level.

## CASE REPORTS

### Case 1

S F Girl born at full term in breech presentation. She gained weight poorly and her development was slow. At the age of 3 months she had a short attack of convulsions. A relapse occurred at 5 1/2 months when she was first seen at the hospital. There were no signs of rickets. Her serum calcium was 2.25 mEq/l and serum inorganic phosphorus 10.0 mg/100 ml. PEG showed paroxysmal dysrhythmias but it has since changed to normal. She had not received the usual vitamin D prophylaxis and was now given 500 000 U vitamin D resulting in normal serum

calcium and inorganic phosphorus levels. The patient was first thought to have spasmophilia. For later data about serum calcium level and therapy see Fig. 1.

At the age of 12 months she had tetany related to an infection with moderate elevation of temperature. She had no teeth and Chvostek's sign was positive. Idiopathic hypoparathyroidism was suspected and the patient was referred to the Department of Paediatrics at Karolinska Institute, Lönnerström's Barnsjukhus, Karolinska Institutet, Stockholm where the diagnosis was verified. For further information see Aperia et al case S F (1).

At 20 months of age she was found to have enamel hypoplasia. Bilateral cataracts were not observed before the age of 4 years and 10 months. She had no radiological signs of rickets intracranial or intrarectal calcium deposits. She had an IQ of 75 (Termin Mental scale) and signs of emotional lability.

Great variations of the serum calcium level occurred and it has been difficult to balance therapy accordingly. At periods when the serum calcium level was high she was tired, lost her appetite and was irritable.

### Case 2

A F Sister of S F born at full term with normal delivery. She was immediately transferred to the department of paediatrics in order to follow the serum levels of calcium and inorganic phosphorus (Fig. 2). Chvostek's sign was positive at low serum calcium levels. Like her sister she was tired, irritable and lost her appetite when hypocalcaemia was present and balanced therapy was difficult to achieve. Signs of tetany only occurred when the patient had hypocalcaemia in connection with fever due to infections. There were no signs of rickets. EEG (when she had normal serum calcium level), X-ray of the skull and eye examinations were normal. Normal eruption of the teeth occurred but there was enamel hypoplasia at the age of 1 year and 10 months. The Liverworth-Howard test was performed at the age of 1 year and 11 months and was positive. During the

This case is further described as case S F by Aperia et al in 1967 (1).

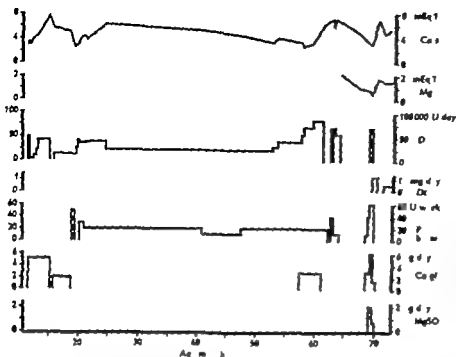


Fig. 1 Case 1 S.F. Survey of changes in serum calcium and serum magnesium in relation to therapy D vitamin D Dx dihydroxycholesterol Ca gluc calcium gluconate

test there were no clinical changes of the blood chemistry. Serum magnesium rose from 1.1 to 1.4 mEq/l. In the control case serum magnesium increased from 1.3 to 1.5 mEq/l.

Both cases have had hypomagnesaemia with lowest serum levels at 0.5 and 0.4 mEq/l respectively determined with the fluorometric method described by Schlicher in 1962 (26). After institution of oral 7 magnesium sulphate solution the serum level of magnesium became normal (see Figs 1 and 2).

They have not had moniliasis and have not shown any evidence of renal dysfunction, malabsorption or associated endocrine disorders.

### FAMILY HISTORY

Convulsions were common in members of this family where the parents are first cousins (see pedigree (Fig.

3) and Table 1). The grandmother of S.F. and A.F. (Fig. 3 II 2) has an abnormal EEG probably due to trauma. The great grandmother and one of her sisters were said to suffer from epilepsy.

Of the mother's siblings five are dead, only one (Fig. 3 III 4) was hospitalized. This girl was born at full term in a normal delivery. At the age of 4 weeks she was fed with cow's milk. Diarrhoea resulted and 2 days later convulsions sometimes associated with cyanosis occurred. These symptoms continued until she died of bronchopneumonia at the age of 2 months. The serum calcium level was never determined. Autopsy revealed numerous glomeruli with fatty globules scattered in the white matter of the cerebrum and in the mesencephalon.

For further information about the relatives see pedigree (Fig. 3) and Tables 1 and 2.

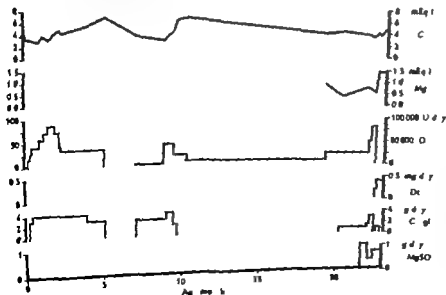


Fig. 2 Case 2 A.F. Survey of changes in serum calcium and serum magnesium in relation to therapy D vitamin D Dx dihydroxycholesterol Ca gluc calcium gluconate

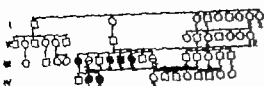


Fig. 3 Pedigree. □ boys ○ girls † died after a period of convulsions \* minor convulsions in infancy • reported cases

## DISCUSSION

The two sisters S F and A F fulfil the criteria of idiopathic hypoparathyroidism. This diagnosis cannot be proved for their mother's siblings but their symptoms are well in accordance with those of idiopathic hypoparathyroidism and in one case the cerebral changes resembles those of earlier described cases (10, 16).

Idiopathic hypoparathyroidism is very rare during the first year of life. Bromsky et al. in 1958 collected 50 cases of idiopathic hypoparathyroidism three of which were younger than 15 months of age (5). In literature 23 cases with hypocalcaemic convulsions due to idiopathic hypoparathyroidism at an age less than one year (early onset) are found (Table 3). Six girls, 16 boys and one patient of unknown sex are reported. There are a few more cases described where the diagnosis is not quite clear (11, 13) and the disease may be more common than actually reported. In 1967 Geer (12) reported a high frequency of parathyroid anomalies in cases of sudden unexpected death in infancy.

Table 1 Individuals in the pedigree (Fig. 3) dead at an early age

No.	Age at death	Case
I 1	9 years	Unknown
I 2	6 months	Infants
I 3	4 weeks	Sudden unexpected death
II 1	19 months	HCT-atrophy
III 1	A few months	Convulsions
III 2	8 days	Immaturity
III 4	1 month	Convulsions
III 5	5½ months	Convulsions
III 6	3½ months	Convulsions

Table 2 Blood chemistry of relatives of cases 1 and 2 Ca and Mg (mEq/l) P (me/100 ml)

For numbers see pedigree (Fig. 3)

No.	Serum levels of		
	Ca	P	Mg
II 2	4.8	3.4	1.6
III 2	4.9	2.6	1.7
III 7	4.1	2.4	1.6
IV 3	5.1	3.1	1.5

Hereditary factors have been found in cases of late onset associated with moniliasis or Addison's disease (9, 19, 22, 30). Of cases with early onset (Table 3) three boys are siblings.

Table 3 Known cases of the early type of idiopathic hypoparathyroidism

No.—reference number T—thyroid P—parathyroid glands a—aplasia h—hypoplasia

No.	Year of publ.	Age at sex	First symptom	T	P	Age at death	Anomalies of other organs
4	1917	♂	4 months	h	h	4 months	Spinal cord
25	1931	♂	6 weeks	h	h	10 weeks	Thyroid gland
25	1932	♂	9 hours	a	a	9 hours	Multiple
24	1938	♂		h		12 weeks	Urinary tract
							Thyroid gland
							Cerebrum
3	1955	♀	8 weeks		a	5 months	
6	1955	♂	7 days				
6	1955	♂	9 days				
23	1956	♀	11 days				Glaucoma
7	1957	♂	3 weeks				
28	1957	♂	2 weeks				
1	1958	♂	Less than 1 year				
17	1959	♂	12 hours	a	a	55 days	
31	1959	♂	A few weeks				
33	1959	♂	25 days				
10	1960	♀	5 days	h	h	11 days	Heart, gall bladder
21	1960	♂	7 weeks				
21	1960	♂	5 days				
34	1962	♂	40 days				
16	1963	♂	27 days	h	h	3 months	Urinary tract
26	1963	♂	3 weeks				
26	1963	♂	1 week				
32	1965	♀	2 hours	a	a	4 weeks	
13	1967	♂	Newborn	a	a	6 months	Heart
Own case		♂	3 months				
Own case		♀	2 days				

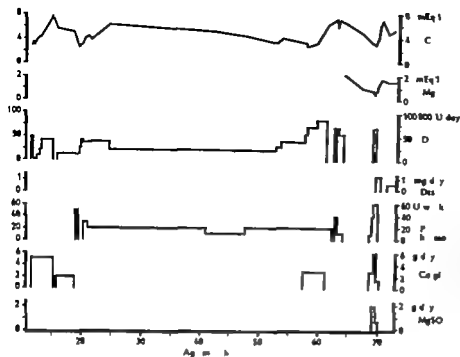


Fig 1 Case 1 S F Survey of changes in serum calcium and serum magnesium in relation to therapy D vitamin D Dts dihydrotachysterol Ca gluc calciumgluconate

test there were no clearcut changes of the blood chemistry. Serum magnesium rose from 1.1 to 1.4 mEq/l. In the control case serum magnesium increased from 1.3 to 1.5 mEq/l.

Both cases have had hypomagnesaemia with lowest serum levels at 0.5 and 0.4 mEq/l respectively determined with the fluorimetric method described by Schacher in 1962 (26). After institution of oral 7 mg magnesium sulphate solution the serum level of magnesium became normal (see Figs 1 and 2).

They have not had moniliae and have not shown any evidence of renal dysfunction, malabsorption or associated endocrine disorders.

### FAMILY HISTORY

Convulsions were common in members of this family where the parents are first cousins (see pedigree (Fig

3) and Table 1). The grandmother of S F and A F (Fig 3 II 2) has an abnormal EEG probably due to trauma. The great grandmother and one of her sisters were said to suffer from epilepsy.

Of the mother's siblings five are dead; only one (Fig 3 III 4) was hospitalized. This girl was born at full term in a normal delivery. At the age of 4 weeks she was fed with cow's milk. Diarrhoea resulted and 2 days later convulsions, sometimes associated with cyanosis, occurred. These symptoms continued until she died of bronchopneumonia at the age of 2 months. The serum calcium level was never determined. Autopsy revealed numerous globose cells with fatty globules scattered in the white matter of the cerebrum and in the mesencephalon.

For further information about the relatives see pedigree (Fig 3) and Tables 1 and 2.

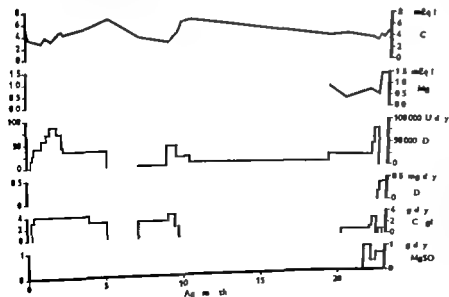


Fig 2 Case 2 A F Survey of changes in serum calcium and serum magnesium in relation to therapy D vitamin D Dts dihydrotachysterol Ca gluc calciumgluconate

- 17 Lobdell D H Congenital absence of the parathyroid glands *Arch Path* 67 412 1959
- 18 MacIntyre I Bore S & Trounstein V A Parathyroid hormone and magnesium homeostasis *Nature* 198 1018 1963
- 19 Morse W I Cochrane W A & Landnagan P L Familial hypoparathyroidism with per seconus anoxia strabismus and adrenocortical insufficiency (A variant of mucopolysaccharidosis) *New Eng J Med* 264 1071 1961
- 20 Packer L Riddle I C Koob S W Conen P H & Fraser H Primary hypomagnesaemia with secondary hypocalcaemia in an infant *Pediatrics* 41 383 1968
- 21 Paden V H True idiopathic hypoparathyroidism in a six bodied recessive trait *Amer J Hum Genet* 12 323 1960
- 22 Quesada M U Leiken S L & Huang W Per seconus anoxia in a young girl associated with idiopathic hypoparathyroidism familial Addison's disease and moniliasis *J Pediatr* 64 241 1964
- 23 Rhyne J L & Carnier F R Idiopathic hypoparathyroidism and bilateral congenital glaucoma in the neonatal period *Pediatrics* 38 433 1966
- 24 Romle R Über den angeborenen Mangel der Epithelkörperchen *Schweiz Med Wochschr* 29 848 1938
- 25 — Über gleichzeitige Missbildungen der bronchio pulmonales Organe und über angeborenen Mangel der Epithelkörperchen *Vierteljahr Arch Path Anat* 263 41 1932
- 26 Schwabacher D The fluorometric estimation of magnesium in serum and in urine *J Lab Clin Med* 54 763 1959
- 27 Smith F G & Zike J Idiopathic hypoparathyroidism in the neonatal period *Amer J Dis Child* 104 182 1963
- 28 Soper R T Mason E E & Buckwalter J A Hypoparathyroidism in children and adolescents *Pediatrics* 20 1097 1957
- 29 Strom L & Wenberg J Idiopathic hypoparathyroidism *Acta Paediatr Scand* 43 574 1954
- 30 Sutphen A Albright F & McCune D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis *J Clin Endocr* 3 625 1943
- 31 Symon L & Bell I M Idiopathic hypoparathyroidism with normal urinary calcium *Brit Med J* 5135 1451 1959
- 32 Tatz L B Zarate Salvador C & Schwartz E Congenital absence of the parathyroid and thymic glands in an infant (III and IV pharyngeal pouch syndrome) *Pediatrics* 39 412 1966
- 33 Vague G Girard R Bernard R Müller H Bernard F Torrains J & Louchet E Hypoparathyroidisme grave de la premiere enfance Evolution clinique et biochimique *Ann Endocr* 0 312 1959
- 34 Yoon Z Garty R Carper J & Benbenisty M Primary hypoparathyroidism in an infant Hypoplastic aberrant parathyroid glands in the pleural submucosa *Amer J Dis Child* 104 307 1962

Submitted Nov 25 1969

Accepted May 4 1970

Barllesken  
Centralasarettet  
Fack  
S-351 01 Vaxjo  
Sweden

Key words Hypoparathyroidism hypomagnesaemia

(6-7) Convulsions in infancy of relatives have been reported (33). These cases could belong to a group classified by Peden in 1960 as idiopathic hypoparathyroidism with early onset inherited as a sex linked recessive trait (21). The present cases cannot belong to this group which consists of boys only. Another group of cases is described with early onset of idiopathic hypoparathyroidism. This group consists of sporadic cases often with multiple congenital anomalies (21). The author's cases do not belong to this group either. Most probably their disease is inherited as a non sex linked trait. It could be an intermediate trait with only minor symptoms in the heterozygote cases. However then one would expect more members of the family to have symptoms.

Hypomagnesaemia associated with the early type of idiopathic hypoparathyroidism has been described in one case (32). Hypomagnesaemia associated with low serum calcium but normal serum inorganic phosphorus levels has also been reported. Therapy with calcium and parathyroid hormone was ineffective in this case (20).

In rats the urinary excretion of calcium and magnesium is decreased at the same time as the phosphate excretion is increased after administration of parathyroid hormone (18). Magnesium deficient rats develop hypercalcaemia and hypophosphataemia and the serum magnesium level is decreased by total parathyroidectomy (14). According to this one would expect hypomagnesaemia to be a sign of idiopathic hypoparathyroidism. This is confirmed by the previous case studied (32) and by the cases of this report. The result of the Ellisworth Howard test in case 2 in contrast to the previous case (32) seems to confirm the effect of parathyroid hormone found in experiments on rats.

### SUMMARY

Two cases of idiopathic hypoparathyroidism are reported and previous cases of the disease with symptoms at an age less than one year are briefly reviewed. Unlike earlier findings there

is evidence of the disease as a non sex linked trait, probably recessive. Both cases had hypomagnesaemia. Administration of parathyroid hormone caused in one patient a rise of the serum magnesium level which has previously been observed in experiments on the rat.

### REFERENCES

1. Aperia A, Broberger O, Bergstrand C G & Zetterstrom R. Renal response to intravenous phosphate load in idiopathic hypoparathyroidism and pseudohypoparathyroidism. *Acta Paediat Scand* 56 357 1967.
2. Bergstrand C G, Ekengren A, Fihlsson R & Hugstert A. Pseudohypoparathyroidism. Familial incidence and comparison with idiopathic hypoparathyroidism. *Acta Endocr* 29 201 1958.
3. Blaim A & Lewicki Z. Zespół tetanyczny oraz ogólny niedorozwój u niemowlęcia z wrodzonym brakiem przytarczyc. *Pediat Pol* 30 923 1955.
4. Bottiger H & Wernstedt W. Beiträge zur Kenntnis der spasmophilen Drüsen IV Mitteilung. Todlich verlaufender Fall von Spasmophilie bei einem Brustkinde mit Anomalien der Thyreus und der Parathyreoidea. *Acta Paediat Scand* 6 373 1927.
5. Bronsky D, Kushner D S, Dubin A & Snapper I. Idiopathic hypoparathyroidism and pseudohypoparathyroidism. Case report and review of the literature. *Medicine* 37 317 1959.
6. Buchs S. Familiärer Hypoparathyreoidismus. *Ann Paediat* 184 364 1955.
7. —. Familiärer Hypoparathyreoidismus. *Ann Paediat* 183 124 1957.
8. Drake T G, Albright F, Bauer W & Castleman B. Chronic idiopathic hypoparathyroidism. Report of six cases with autopsy findings in one. *Ann Intern Med* 12 1751 1939.
9. Edgren G. Fall av idiopatisk tetani. *Läkarskriften* 50 1722 1953.
10. Firber S & Wawter G F. Clinical pathological conference. *J Pediat* 56 690 1960.
11. Forbes G B. Clinical features of idiopathic hypoparathyroidism in children. *Ann N Y Acad Sci* 64 432 1956.
12. Geertinger P. Sudden unexpected death in infancy. With special reference to the parathyroids. *Pediatrics* 39 43 1967.
13. Harrison H E. Idiopathic hypoparathyroidism. *Pediatrics* 17 442 1956.
14. Henton F W. The parathyroid glands and magnesium metabolism in the rat. *Clin Sci* 28 543 1965.
15. Huber J, Chotnoky P & Zoetboot H E. Congenital aplasia of parathyroid glands and thymus. *Arch Dis Child* 42 190 1967.
16. Kunstader R H, Tanman W O F & Cornblath M. Idiopathic hypoparathyroidism in the newborn. *Amer J Dis Child* 105 499 1963.

- 7 Lobbell H H Congenital absence of the parathyroid glands *Arch Path* 67 412 1959
- 8 MacIntyre I Bone & Troughton V A Parathyroid hormone and magnesium homeostasis *Nature* 198 1058 1963
- 9 Moore W I Cochrane W A & Landrum P L Familial hypoparathyroidism with perioral acroecia, diarrhoea and adrenocortical insufficiency (A variant of mucoviscidosis) *New Eng J Med* 264 1021 1961
- 10 Palmer L Radde I C Koob S W Cohen P E & Fraser D Primary hypomagnesaemia with secondary hypocalcaemia in an infant *Pediatrics* 41 345 1968
- 11 Peden V H True idiopathic hypoparathyroidism is a sex-linked recessive trait *Amer J Hum Genet* 12 323 1960
- 12 Quete M C Leikin S L & Hung W Perioral acroecia in a young girl associated with idiopathic hypoparathyroidism familial Addison's disease and moenhaus *J Pediatr* 64 241 1964
- 13 Rhyne J L & Carraker F R Idiopathic hypoparathyroidism and bilateral congenital glaucoma in the neonatal period *Pediatrics* 18 442 1956
- 14 Roske R Über den angeborenen Mangel der Epithelkörperchen *Schweiz Med Wochschr* 29 848 1938
- 15 — Über gleichzeitige Missbildungen der brüchigen Organe und über angeborenen Mangel der Epithelkörperchen *Fortschr Arch Path Anat* 283 41 1932
- 16 Schachar D The fluorometric estimation of magnesium in serum and in urine *J Lab Clin Med* 54 763 1959
- 17 Smith F G & Zile J Idiopathic hypoparathyroidism in the neonatal period *Amer J Dis Child* 103 182 1963
- 18 Soper R T Mason E E & Bockwaller J A Hypoparathyroidism in children and adolescents *Pediatrics* 20 1097 1957
- 19 Strom L & Winberg J Idiopathic hypoparathyroidism *Acta Paediatr Scand* 43 574 1954
- 20 Strupis A Albright E & McCone D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moenhaus *J Clin Endocr* 5 625 1943
- 21 Symon L & Bett J M Idiopathic hypoparathyroidism with normal urinary calcium *Brit Med J* 5135 1451 1959
- 22 Taitz L S Zarate Salvador C & Schwartz E Congenital absence of the parathyroid and thyroid glands in an infant (III and IV pharyngeal pouch syndrome) *Pediatrics* 38 412 1966
- 23 Vague G Girard R Bernard R Müller B Bernard P Torresani J & Loocher E Hypoparathyroïde grave de la première enfance Évolution clinique et biochimique *Ann Endocr* 20 312 1959
- 24 Yous Z Garty R Casper J & Benbarat M Primary hypoparathyroidism in an infant Hypoplastic aberrant parathyroid glands in the pharyngeal submucosa *Amer J Dis Child* 104 307 1962

Submitted Nov 25 1969

Accepted May 4 1970

Barnhoken  
Centralbasarett  
Fack  
S 351 01 Växjö  
Sweden

Key words Hypoparathyroidism hypomagnesaemia



# NEW BOOKS RECEIVED

- M Adinolfi (ed) *Immunology and development* Clinica in Developmental Medicine no 34 185 pp illus W Heinemann Medical Books Ltd London 1969 63s
- W I Nelson V C Vaughan III & R J McKay (eds) *Textbook of Pediatrics* 1590 pp 527 illus W B Saunders Co Ltd Philadelphia London and Toronto 1969 £9 3s
- R J Robinson (ed) *Brain and early behaviour* 374 pp illus Academic Press Inc London 1970 100s
- M Joseph & C Dawbarn *Measurement of the Face: A study in Down's syndrome* 114 pp illus S I M P Research Monograph No 3 W Heinemann Medical Books Ltd London 1970 30s
- G Joppich & H Wolf (eds) *Metabolism of the newborn child* 448 pp illus Hippokrates Verlag Stuttgart 1969 DM 75
- N Finnse *Nær med cerebral parese* 192 pp illus Borgens Forlag København 1970 Dkr 36 00
- Y Manuel J H Revillard & H Beteul (eds) *Proteins in normal and pathological urine* 354 pp illus S Karger Basel 1969 DM 90
- L Tuft & H L Moeller *Allergy in children* 561 pp illus W B Saunders Co Ltd Philadelphia London and Toronto 1970 £8 6s
- P G Jones (ed) *Clinical paediatric surgery. Diagnosis and management* 528 pp illus J Wright & Sons Ltd Bristol 1970 120s
- Report of a WHO Scientific Group *Genetic factors in congenital malformations* 42 pp World Health Organization Technical Report Series 1970 no 438 US\$1 00
- A Ambrose (ed) *Stimulation in early infancy* 289 pp Academic Press London and New York 1970 £4 00
- P C Arquembourg J E Salvaggio & J N Bickels *Primer of immunoelectrophoresis* With interpretation of pathologic human serum pattern 84 pp 117 illus S Karger AG Basel München Paris and New York 1970 DM 33
- W R Aykroyd *Conquest of deficiency diseases Achievements and prospects* 98 pp World Health Organization Geneva 1970 US\$3 00
- G Debrun & C Gasquet *Explorations vasculaires des tumeurs abdominales de l'enfant* Monographies des Annales de Radiologie A 92 pp 44 illus Expansion Scientifique Française Paris 1970 I 40
- S S Gellis & B M Kagan (eds) *Current pediatric therapy—A* 1077 pp W B Saunders Co Ltd London 1970 £11 9s 6d
- O Grosser & R Ortman *Grundriss der Entwicklungsgeschichte des Menschen* 207 pp 700 illus Springer Verlag Berlin Heidelberg and New York 1970 DM 28
- R Frey & Th Brumann (eds) *Nephrologie im Kindesalter II Pädiatrische Fortbildungskurse für die Praxis* 114 pp illus S Karger AG Basel München Paris and New York 1970 US\$7 20
- W A Fuchs J W Davidson & H W Fischer *Recent results in cancer research* Vol 23 *Lymphographic in cancer* 294 pp 189 illus Springer Verlag Berlin Heidelberg and New York 1969 DM 76
- H Salem & D M Ariado (eds) *Antihistaminic agents* Vol I II III International Encyclopedia of Pharmacology and Therapeutics Section 27 Pergamon Press Oxford New York and Toronto 1970 £12 US\$32 00
- J Huntington K A Huter & E Silber (eds) *Perinatal medicine* 1st European Congress Berlin 299 pp 197 illus Georg Thieme Verlag Stuttgart 1970 DM 37
- S Jolkowski (ed) *Cystic fibrosis and related human and animal diseases* 240 pp Gordon and Breach Science Publ Inc New York 1970 US\$19 50
- P Kreppler *Grundlagen und Fortschritte der Leukämiebehandlung beim Kinde* 158 pp illus Ferdinand Enke Verlag Stuttgart 1970 DM 36
- F S Perlman (ed) *Recent research on the retina* 178 pp British Medical Bulletin Vol 26 No 2 London 1970 £2
- E Rannmar & H Renling *Det idealiska summern let. En studie hur neurosedivitragedin påverkar ödet* 112 pp Raben & Sjögren Stockholm 1970 Swkr 9 30
- G Rooth *Clinical acid base and electrolyte balance* 80 pp Studentlitteratur Lund 1970 Swkr 14 30
- H G Schwarzscher & U Wolf (ed) *Methoden in der medizinischen Cytogenetik* 186 pp illus Springer Verlag Berlin Heidelberg and New York 1970 DM 48
- k Tóth *The epidemiology of dental caries in Hungary* 283 pp Akadémiai Kiadó Budapest 1970 US\$10 00
- D H M Woolham (ed) *Advances in teratology* Vol 4 240 pp illus Logos Press Ltd London 1970 100s
- Ulund Forsnäs till beskrivning av några utvecklingsproblem SIDA Stockholm 1970
- Troubles vasculaires de l'enfant et de l'adolescent* Colloque de la Société Française de Phlébologie 172 pp illus Expansion Scientifique Française Paris 1970 F 35

# BOOK REVIEWS

II Heyck & G Landahn *The progress of dystrophy*  
Acta Myopathica Springer Verlag Berlin Heidelberg  
New York 1969 436 pp 141 ill DM  
118 —

The book gives an excellent review both of historical and of recently discovered facts about muscular dystrophy. It is limited to progressive muscular dystrophy; other muscle diseases are mentioned only as differential diagnostic possibilities.

In chapter 1 the clinical classifications of muscular dystrophy and the histopathological findings are discussed. The history of the condition is thoroughly reviewed starting with the first known case of muscular dystrophy apparent from an Egyptian picture roughly 3300 years old. One can agree with the authors' statement that at present without knowledge of the biochemical background of the various types of muscular dystrophy a classification should preferably be based on genetic grounds and also with their own objection that this is usually impossible as reality. Thus their own classification like most other usable classifications is based on a mixture of clinical and genetic features. The description of the various types of muscular dystrophy is clear; the authors' own personal experience with a large material of muscular dystrophy makes this part of the chapter particularly valuable. The discussion of the findings on routine histopathological examination of muscle specimens adds little to our previous knowledge.

In chapter 2 Elizabeth Freund-Molbert gives a beautiful description of the electromyoscopic findings in muscle diseases.

Chapter 3 reviews in an excellent way the known facts about the metabolic disorders present in muscular dystrophy concentrating on the authors' main interest: the abnormal enzyme activity in blood and muscle tissue. The variation of the enzymes according to age and their distribution within the muscle cell and between various muscles in health and disease are described in great detail. This work represents a fascinating and important contribution to our basic knowledge about the muscle enzymes. The number of enzymes studied is overwhelming. One only wishes a more profound discussion about the activity in the series of the isoenzymes of lactate dehydrogenase as the examination of these isoenzymes seems in some patients and in some healthy persons to be as important as the study of creatine kinase. It should also be pointed out that the whole chapter is concerned only with the biochemical aspects and that biochemical studies of muscle tissue an important contribution to our knowledge of muscle diseases is mentioned only briefly.

Chapter 4 is concerned with the distal types of muscular dystrophy; chapter 5 with ocular types, chapter 6 with congenital forms and chapter 7 with monogenic myopathies. In these chapters the authors review the present knowledge and the controversial opinions.

In chapter 8 Herbert Müller-Stephann & Peter Schmidt-Peter describe the possibilities of physical therapy and orthopedic measures in muscle diseases. One can fully agree with these authors that physical therapy is extremely important to help the patient although it does not cure the disease and that immobilisation will lead to a rapid deterioration. A little more debatable is the generous recommendation of various splints as these may cause the patient pain and discomfort and of orthopedic operations to relieve contractures; the gain of these operations is not evaluated in relation to the possible loss caused by the necessary immobilisation afterwards.

The discouraging result of all medicamentous therapy tried is reviewed in chapter 9. The final chapter is written by Dietrich Toennis & Manfred Wolter. It gives a clear description of the performance of electromyography and interpretation of the findings in adults without adding anything new.

This well-planned book can be recommended to everybody interested particularly in the clinical and the biochemical aspects of muscular dystrophy and in the possibilities of physical and orthopedic therapy. The thorough penetration of the historical background should be particularly stressed; the book contains about 1850 references covering almost 70 pages.

Ingrid Guntzberg

D Janz *Die Epilepsien: Ihre Pathologie und Therapie* G Thieme Verlag Stuttgart 1969 554 pp ill., DM 118 —

Dieter Janz's monograph is a milestone in epileptology comparable to the books by Prof. Feldt and Jasper and that by William Lennox.

Janz dedicates his book to Paul Vogel, eminent stylist among German neuropsychiatrists, to Hugh Jackson, the thinker among the ancestors of neurology and to Dostoyevsky, the explorer of the consciousness of the epileptic.

It is evident that Janz himself is an excellent stylist, a diligent explorer of body and mind and an imaginative thinker.

The book is based on vast own patient material that for many years has been subject to veritable investigations by Janz and his many fellow workers at the Heidelberg School.

Janz submits a nosographical classification of the epilepsies that seems convincing and useful. It is based on a meticulous description of the various types of fit and a correlation with the electro-encephalographic findings, the age at onset, the course of the disease and the influence of the sleeping/waking cycle. The most clearly defined groups are the fit in infantile and juvenile forms of petit mal epilepsy. The terminology will probably give rise to argument. In infantile spasms, myoclonic attacks, epilepsy and myoclonic jerking are all called petit mal, a term which in Scandinavian and English speaking countries is reserved for pyknoleptic petit mal.

Each chapter starts with a carefully assembled and very interesting list of synonyms elucidating the scientific history of epilepsy and preventing the reader from being confused. It also shows as does the whole book, Janz's intimate knowledge of the literature.

The text is abundant with the evidence of detailed clinical experience. Even mistakes of epileptology will find surprising observations again and again.

Faced with controversial problems Janz is not the type to leave them unsolved. He prefers to venture upon provocative hypotheses arousing the interest of his reader.

A few critical remarks

Janz apparently is not fond of statistics. Some times the reader doubts how safe the basis of the conclusions might be. The importance of dosing anti-epileptic drugs according to the weight is not mentioned. Control of treatment by testing the concentration of the drug in the serum is not touched upon either.

The leisurely style and the many repetitions make the book as easily readable as a novel. The reviewer considers this an pedagogical asset.

The reference list is very comprehensive, the subject index very differentiated.

Janz's book should be read by every epileptologist including every neuropediatrician. The young doctor showing interest in any aspect of epilepsy should be referred to this book in the first place.

Mogens Lund

N. Lloyd Rusby (ed.) *New ideas in asthma and its management*. The chest and heart association. Health Horizon Ltd. London 1969. 56 pp. 10s.

Papers read at a symposium in London 1969 over asthma and its management have been collected in a small well worth reading volume. In a controlled trial over the effect of Intal (sodium chromoglycate) in pollen allergic asthmatics A. W. Frankland did not find a significant difference between the control group and the Intal group. However he is not negative to the drug: we can be certain that further uses will be found for disodium chromoglycate. D. G. Wraith reports results from investigations of dust allergy and mites. The observations set forth by the Dutch group (Voorspoort and co-workers) that mites from house dust may frequently cause allergic skin

reactions are confirmed. Some effects of hyposensitization have been found in asthmatics in a controlled trial by G. P. Maher Loughnan.

Leslie Scott reports preliminary results from a follow up investigation of boys who have passed a Residential Boarding School for asthmatics. The group was a highly selected one as the school receives mainly boys who have failed to respond to conventional treatment. With this in mind the results must be regarded as remarkably good. 41 of 47 boys followed up for three years after discharge from the school were leading a normal life or had symptoms which did not cause interference in the process of education or earning a living.

The inhalant allergens in bronchial asthma in the U.A.R. have been studied by A. El Hefay and employment of asthmatics by Helen Zach. Two papers concern treatment with bronchodilator aerosols (by I. W. H. Grant and P. Heat). The title of one of them is "Sudden Death in Asthma". Associated with the rise in the death rate there has been a parallel rise in the use of aerosols. After the Dunlop Committee working in 1967 and the decline in the sales of these aerosols the mortality has also fallen.

Bengt Kjellman

D. Koldovsky: *Development of the Functions of the Small Intestine in Mammals and Man*. 204 pp.illus. S. Karger, Basel. New York 1969. Dfl. 55.—

In the last ten years there has been an increasing interest to study the development of the morphology and function of the gastrointestinal tract. The Laboratory of Developmental Nutrition in Prague under Dr. O. Koldovsky is one of the leading centres for this subject. In this book Dr. Koldovsky has in an excellent way summarized his and his collaborators' extensive studies in this field and furthermore collected all information available in literature today.

The book can be divided in two parts. The one deals with the development in different mammals and the other with the human fetus. For natural reasons the first part is the most extensive. The changes of macro- and micro-structure of small intestine, the development of motor activity as well as the absorption of water, ions and vitamins are discussed. A relatively large part is devoted to the digestion and absorption of carbohydrates where the existence of several beta-galactosidase activities has been especially discussed. The development of digestion and absorption of fats has only been studied to a relatively small extent while more information concerning the proteins and amino acids is available. One of the most interesting questions in this field is the problem of regulation of protein transfer through the mucosal cells occurring in the neonatal period in several species. The developmental changes of other enzyme activities as alkaline phosphatases and beta-glucuronidase are also discussed. The book ends with a long and apparently complete reference list.

Knowledge of developmental changes of the function of the small intestine is not only of great physiological interest but it also contributes to our understanding of pathological conditions due to enzyme defects. Therefore this book is highly recommended to the gastroenterologically interested paediatrician.

*Tor Lindberg*

G E W Wobstenholme & M O Connor (eds) *For and Against: A Ciba Foundation Symposium 1 & A Churchill Ltd London 1969 326 pp 70s*

Data, development, the mammalian organism passes through a number of critical periods—at implantation, the timing of organogenesis at birth and after birth during the establishment of behavioural patterns—periods which pass never to return. Failure to make its transition at the right time is crippling or lethal. The conclusions concerned are fascinating, and difficult to unravel. Investigators separated not only by distance but even more by discipline are working on these problems. With the purpose of exploring different approaches to the study of fetal autonomy a Ciba symposium was held in London in December 1968 under the chairmanship of Dr G S Davies. The proceedings of this symposium are published in this volume.

R. V. Short is searching for the answer to the question: How soon does an animal know that it is pregnant? Is implantation an essential prerequisite for its sustained recognition of pregnancy? The human and the monkey embryo implant and secrete a chorionic gonadotropin that may be heterotropic before the first sexual maturation period and thereby causing the first sustained recognition of pregnancy. In the horse however pregnancy may even be recognised while the egg is still in the Fallopian tube. Another interesting problem considered is the immunological relation between mother and foetus. Why does the pregnant woman tolerate her baby which brings an allograft should be incompatible with her and so be rejected? According to C. A. Currie the mother's immune mechanisms are controlled by fetal antigen from the blastocyst at the time of implantation and later by the trophoblast. The maternal antibodies will recognise any fetal structure when reach. This will be so the trophoblast being the only tissue in direct contact with maternal cells but as the trophoblast is of low antigenicity it is not destroyed by the antibodies. The antibodies adsorb to the trophoblast surface and inhibit cellular immune reactions both maternal-to-fetal and fetal-to-maternal. Besides this the trophoblast acts as barrier by effectively limiting the number of cells passing between the two circulations.

A number of papers are concerned with the hormonal balance and steroid metabolism in the foetus. The development of the fetal lung in different species is discussed by R. E. Pattle with interesting aspects on the respiratory distress syndrome of the human newborn as the comforting fact that many foals

suffering from the "barker" syndrome (i.e. RDS of foals) may develop into good racehorses. The course and distribution of the fetal circulation and fetal blood gas homeostasis is described in an excellent paper by G. S. Davies while H. Tuchman Duplexian reviews the reactions of the fetus to drugs taken by the mother. The discovery that in the mouse a genetic ataxia can be prevented by giving the pregnant animal a supplement of manganese reveals new fields of research and provides hope of finding new ways to prevent congenital malformations.

The different papers and especially the discussions following each paper presented in this volume should be of great interest to everybody seeking the integrated function of the whole from the mechanism which regulates it and their limits of tolerance on which fetal autonomy depends.

*Vida W. S. S. S. S.*

J. L. Melnick (ed) *Progress in Medical Virology* vol 11 S. Karger Basel and New York 1969 469 pp US \$21.35

The 1969 issue of *Progress in Medical Virology* like many previous volumes is not primarily devoted to subjects in the field of paediatrics but still it is of great general interest to everyone working within or on the border of infectious diseases. As usual topics of great current virologic interest have been chosen. Some subjects are mainly of basic virological or methodological interest. So are the informative survey by Darlington & Blom on recent work on the course of herpes virus, the interesting paper by Dougherty & Di Stefano on cytopathism of leukaemia viruses and the excellent article by Howe, Morgan & Him on recent virologic applications on ferritin conjugates. These three papers are all illustrated with beautiful electron micrographs. Horn & Tyrrell summarise the use of organ cultures in virology.

Some articles represent a more clinical approach. These include the interesting survey of Hermon on applications of infectious nucleic acids in diseases—clarification in this field may possibly develop in the expanding investigations of "slow viruses". Perva gives a distinguished review on the antigenic spectrum of influenza. The mechanism of antigenic variation and the origin of virus subtypes is still poorly understood. Further knowledge may be gained through search for new influenza viruses in animals. Za Rham's comprehensive article on the association of a newly recognized papovavirus with a human demyelinating disease (progressive multifocal leukoencephalopathy PML) is of interest to the clinician as is the informative survey by Haughton & Nash on normally occurring transposition elements along with data on antigenic changes in neoplasms. The understanding of control of cancer may possibly come from further study of tumor immunology.

Some subjects are of explicit clinical—and also paediatric interest e.g. the comparison of two attenuated rubella virus vaccines one developed in the

USA and described by Parkman & Meyer and one in Belgium and reported by Huygelen Peetermans & Pinzie. Problems in immunization against mumps are discussed by Deinhardt & Shramek. An exteriorizing interesting report is published by Hope Simpson & Higgins on a combined field and laboratory study of acute respiratory illnesses occurring during 5 years in their practice population of 3500 persons. This investigation makes a real contribution to the understanding of ways in which the common respiratory viruses participate in the recurrent pattern of human illnesses. It is somewhat to be regretted that regular serologic investigations and the use of controls were not included in this otherwise excellent study. The work is an outstanding example of the opportunities for investigative work open to the interested physician in general practice.

In a traditional final section of the volume the Editor presents a brief note on the development of the classification of viruses.

Curt Carlström

I. S. Schulman (ed.) *Advances in pediatrics* vol. 16. Year Book Medical Publishers Inc. Chicago Ill. 1969. 469 pp. US \$15.00.

The 1969 volume of *Advances in pediatrics* appears under the auspices of a new editor and a new editorial board. Since 1947 this well-known series has been guided by Dr Samuel Z. Levine, with volume 16 the leadership passes to Dr Irving Schulman. In his preface the new editor expresses the hope that the high standards which have been established during the past 22 years will be maintained. The present volume leaves no doubt that these intentions have been realized. Thirteen articles of a very high quality are presented, about double the average number of preceding volumes dealing with diverse subjects of modern pediatrics. Space prohibits a complete review of the content but it may be mentioned that articles like 'Newer viral exanthems' by James D. Cherry and 'Advances in pediatric ophthalmology' by Mrs Kaplin in an excellent way summarize information which for the average pediatrician must be hard to collect from different sources.

C. C. Bergstrand

C. N. Burnard & V. Schrire. *Die Chirurgie der Herzmishildung*. Springer Verlag Berlin Heidelberg and New York 1969. 152 pp. DM 12.80.

This book is written by the well-known heart team Burnard & Schrire and deals with the problems most often met by general pediatricians. Young cardiologists and thoracic surgeons will get good information from this book as it is concentrated and easily read.

The authors discuss the six most common congenital heart diseases (persistent ductus arteriosus, coarctation of the aorta, atrial septal defect, ventricular septal defect, Ebstein's tetralogy and pulmonary stenosis with intact ventricular septum).

For every condition a clinical description, relevant investigations, indication for operation and method of operation are given.

The book is supplemented with excellent photographic and schematic illustrations.

The preferred age for operation is between 5 and 10 years for all these congenital diseases as the operative mortality is lowest at that age. The mortality for PDA is 2.6%. For coarctation of the aorta and secundum atrial septal defect 0%. Burnard & Schrire have 35 cases of primum defects with 2 deaths. In 74 cases with VSD 6 patients died and in the cases with pulmonary stenosis 38 had valvular stenosis (2 died) and 10 had infundibular stenosis (1 died). The first 100 cases of Ebstein were operated upon with a mortality of 12% (this is now lowered to 2%).

These surgical results are as expected excellent and underline the importance of early examination of these children. If the clinical examination gives suspicion of heart disease the procedure is early referral of the child to a team of cardiologists and cardiac surgeons for further investigation and evaluation for surgery.

I can recommend this book to doctors concerned with the welfare of children.

P. J. J. Janssen

# General Index

## Authors Index

- Achel R A 211  
 Andersen J B 699  
 Antikainen I and Sonda S 537  
 Aperia A et al 605  
 Asvora A L et al 177 385  
  
 Bakken A F 148 153  
 Balfanz A et al 497  
 Barak Y 363  
 Barnes R D et al 403  
 Beckman G et al 205  
 Bengtsson B et al 197  
 Berg U et al 1  
 Berglund O 664  
 Bergstrand C G and Nilsson K O 427  
 Bernal I et al 58  
 Berber C P et al 697  
 Burkbeck J A 505  
 Bjernstol A et al 249  
 Brodehl J et al 241  
  
 Carlstrom G and Jalling B 303  
 Chedd M A et al 134  
  
 Dahl G 65  
 Dahlqvist A et al 621 669  
 Donckerwolcke R A M G et al 399  
  
 Ekstrand H et al 33 297 349  
 Elomaa R 147  
 Ericson N O et al 487 491  
 Ericson Y and Ruben U 424  
 Eriksson M 517  
  
 Fergelson J et al 539  
 Feldman W et al 52  
 Fjord Christensen M et al 613  
 Fjord Christensen M and Therkelsen A J 706  
 Flisrud L B and Orstavik I 536  
  
 Gaburro II et al 53  
 Galal O M et al 137  
 Ghaderi H et al 233  
 Goedde H W et al 83  
 Grant C A and Robertson B 208  
 Grant D B et al 432  
 Gruhn C M et al 221  
  
 Hagberg B and Westphal O 323  
 Hagberg B and Sillanpaa J 13  
 Hagberg B et al 545  
 Hammarström L et al 631  
  
 Haldkowski B et al 44 164  
 Hall B 199  
 Halvorsen S et al 28  
 Hilgartner M W et al 409  
 Holmberg L 558  
 Hoyernal H M et al 185  
  
 Isler N J 351  
  
 Jalling B and Carlstrom G 303  
  
 Kayser A and Öckerman P A 214  
 Keritzky D et al 291  
 Karlberg P and Senterre J 653  
 Kessel P and Stroder J 288  
  
 Lankowsky P and McGoldrick K E 711  
 Laron Z et al 317  
 Lebesch E et al 217  
 Lempert R and Liseqvist B 377  
 Leonard H G and Penningstorff H 565  
 Leonard H G 572  
 Lervon H et al 648  
 Liseqvist B and Lempert R 377  
 Lindblad B S 13  
 Lindblad B S et al 21  
 Lunde L M et al 169  
 Linderholm R et al 141  
 Lundquist B and McCusker G W 74  
 Lund H T et al 582  
 Lundh B et al 121  
 Lundquist A and Öckerman P A 293  
  
 McGoldrick K E and Lankowsky P 711  
 McCusker G W and Lundquist B 74  
 Moc P J 49 381  
  
 Nielsen A and Oyer J 416  
 Nilsson E 715  
 Nilsson I M and Öckerman P A 127  
 Nilsson K O and Bergstrand C G 427  
  
 Olsgård R and Svanerholm L 637  
 Olm P 343  
  
 Penningstorff H and Leonard H 565  
 Polak J and Zilka J 391  
  
 Ruben U and Ericson Y 424  
 Richardson S A 265  
 Robertson B and Grant C A 208  
 Rikhi N C R and Vapaavuori E K 353  
  
 Samuelson G 94  
 Saxstad J et al 676

USA and described by Parkman & Meyer and one in Belgium and reported by Huygelen Peetermans & Prinze. Problems in immunization against numps are discussed by Deinhardt & Shramek. An extraordinary interesting report is published by Hope Simpson & Higgins on a combined field and laboratory study of acute respiratory illnesses occurring during 5 years in their practice population of 3 500 persons. This investigation makes a real contribution to the understanding of ways in which the common respiratory viruses participate in the recurrent pattern of human illnesses. It is somewhat to be regretted that regular serologic investigations and the use of controls were not included in this otherwise excellent study. The work is an outstanding example of the opportunities for investigative work open to the interested physician in general practice.

In a traditional final section of the volume the Editor presents a brief note on the development of the classification of viruses.

Gun Carlström

I Schulman (ed) *Advances in pediatrics* vol 16 Year Book Medical Publishers Inc Chicago Ill 1969 469 pp US \$15 00

The 1969 volume of *Advances in pediatrics* appears under the auspices of a new editor and a new editorial board. Since 1947 this well-known series has been guided by Dr Samuel Z. Levine with volume 16 the leadership passes to Dr Irving Schulman. In his preface the new editor expresses the hope that the high standards which have been established during the past 22 years will be maintained. The present volume leaves no doubt that these intentions have been realized. Thirteen articles of a very high quality are presented about double the average number of preceding volumes dealing with diverse subjects of modern paediatrics. Space prohibits a complete review of the content but it may be mentioned that articles like 'Newer viral exanthems' by James D. Cherry and 'Advances in pediatric ophthalmology' by Max Kaplan in an excellent way summarize information which for the average paediatrician must be hard to collect from different sources.

C. G. Bergstrand

C. N. Barnard & V. Schrire *Die Chirurgie der Herzmissbildung* Springer Verlag Berlin Heidelberg and New York 1969 152 pp DM 12 80

This book is written by the well known heart team Barnard & Schrire and deals with the problems most often met by general paediatricians. Young cardiologists and thoracic surgeons will get good information from this book as it is concentrated and easily read.

The authors discuss the six most common congenital heart diseases (persistent ductus arteriosus coarctation of the aorta atrial septal defect ventricular septal defect Fallot's tetralogy and pulmonary stenosis with intact ventricular septum).

For every condition a clinical description relevant investigations indication for operation and method of operation are given.

The book is supplemented with excellent photographic and schematic illustrations.

The preferred age for operation is between 5 and 10 years for all these congenital diseases as the operative mortality is lowest at that age. The mortality for PDA is 2.6 %. For coarctation of the aorta and secundum atrial septal defect 0. Barnard & Schrire have 35 cases of primum defects with 2 deaths. In 74 cases with VSD 6 patients died and in the cases with pulmonary stenosis 38 had valvular stenosis (2 died) and 10 had infundibular stenosis (1 died). The first 100 cases of Fallot were operated upon with a mortality of 12 %, this is now lowered to 2 %.

These surgical results are as expected excellent and underline the importance of early examination of these children. If the clinical examination gives suspicion of heart disease the procedure is early transferring of the child to a team of cardiologists and cardiac surgeons for further investigation and evaluation for surgery.

I can recommend this book to doctors concerned with the welfare of children.

P. Lauridsen

Galectinase deficiency 669

Gemra globulins

spina bifida cystica 134

Gastrointestinal tract

anorectal stress 473

chloride diarrhea 441

cholestasis 443

colic due to albumin metabolism 452

colic due to antibodies in 453

colic due to diagnostic criteria 461-463

diarrhoea chronic treatment of 446

diarrhoea sugar induced 455

dipeptidase 443 456 671

duodenal obstruction 448

enterokinase deficiency 456 457 458

gastroenteritis rare E. coli type 460

glucose galactose malabsorption 74 214

gluten intolerance frequency of relapses 481

gluten intraduodenal instillation of 463

haemobilia traumatic 471

hyperbilirubinaemia prepartal 451

immunohistochemistry intestinal mucosa 447

immunologic deficiency 447

intestinal biopsy 459

intestinal flora, malabsorption 449

intestinal malabsorption 773

intestineception 473

jejunal mucosa malnutrition 58

lactic acid faeces 448

lactose intolerance 451

lipids in serum 445

Low's syndrome 453

malabsorption lesions in 446

malnutrition hormonal interrelationships 459

metabolic transport 450

milk allergy lymphocyte transformation 449

morphology bowel 448

neonatal changes endocrine disorders 443

neonotomosis liver function bile tract 539

pancreozymin effect of 444 692

portal hypertension 472

protein intolerance 451

protein losing gastroenteropathy 217

protein malabsorption diagnosis of 454

regional enterocolitis 473

secretin effect of 444 692

sucrose isomaltase localization of 460

triglycerides long chain hydrolysis rate 444

Genital organs

clitoris correction of hypertrophy 467

anterior surgical treatment 465 466

Winkelman's operation 471

Glycogen storage diseases

genetic studies 5 9

hepatomegalic forms bleeding disorders 177

hyperkalemia in 701

Haemolytic uraemic syndrome 347

Hand-Schüller-Christian's disease 343

Haptoglobins

malignant disease 381

Hypercalcaemia

fat necrosis subcutaneous 228

Hyperparathyroidism primary 249

Hyperviscosity Leach-Nyhan disease 233

Immunology

cellular immunity deficient in ataxic diplegia 545

C-reactive protein in infants III 676

deficiency intestinal mucosa 447

Infants

C-reactive protein 25

jejunal mucosa in malnutrition 58

Infections

adenovirus 310

cytomegalovirus 303

inclusion body disease 227

meningitis haemophilus influenzae amplicon 211

neonatal meningitis 46 427

neonatal osteomyelitis 227

neonatal pneumonia 226

toxoplasmosis congenital generalized 103

urinary tract renal function 605

Kallmann's syndrome 229

Leach-Nyhan disease 233 259

Low's syndrome 453 459 631

Malabsorption

diagnosis of protein malabsorption 454

dipeptidase disaccharidases 621

glucose galactose 74 214

gluten intolerance frequency of relapses 481

intestinal 273

intestinal flora 449

lesions in 446

renal glucosuria 456

Malformations

cerebral malformations thrombocytopenia 185

ectodermal dysplasia III

ectodermal vesicle syndrome 469

hydrocephaly 105

Low's syndrome 248

major and minor malformations newborn 297

myelodysplasia syndrome 467

myelomeningocele anal continence 491

myelomeningocele bladder function 487

neural naevi 416

renal dysplasia I

sulphydryl induced damage 517

spina bifida cystica 134

telangiectases 416

uremia 469

Malignant diseases 228

Malnutrition

hormonal interrelationships 459

jejunal mucosa in 58

treatment 446

Maple syrup urine disease 83

Mastocytoma 358

Metabolism

amino acids hereditary fructosemia 141 227 432

amino acids phenylketonuria 241

depot fat fatty acid composition of 505

fatty acids plasma 637

glycogen storage disease bleeding disorders 127

hypercalcaemia sarcoedema 582



Seuthe J and Karlberg P 653  
 Serik O 340  
 Sillanpää M and Hagberg B 328  
 Simola S et al 310  
 Simola S and Anttilainen I 587  
 Sommerschild H C et al 513  
 Stalsberg H 685  
 Stroder J and Kasai P 288  
 v Studnitz W 80

Tenconi R and Zaccello F 88  
 Therkelsen A J and Fjord Christensen M 706

De Vaan G A M and Voors G P 90  
 Vapaavuori E A and Riihi N C R 353  
 Vest M et al 681  
 Vestermark S 435  
 Viikari J K et al 273 481  
 Vitek B et al 701  
 Voors G P and De Vaan G A M 90  
 Waaler P E et al 529  
 Westphal O and Hagberg B 323

Zaccello F and Tenconi R 88  
 van der Zee S P M et al 259  
 Zicka J and Polak J 591  
 Zoppi G et al 692

Öckerman P A and Nilsson J M 127  
 Öckerman P A and Kauppi A 214  
 Öckerman P A and Lundquist A 293  
 Östlavik I and Flugstad L B 536  
 Orier J and Nielsen A 416

## Subject Index

### Acid-base balance

exchange transfusion 111  
 newborns

### Amino acids

branched-chain ketoaciduria 83  
 cystathioninuria neuroblastoma 80  
 cystinosis treatment of 613  
 fructosemia hereditary 141 432  
 methylmalonic acidemia 28  
 plasma levels in newborns 13 21

### Amniotic fluid

chemical components 111

### Asphyxia

EEG 110  
 hyponatremia in 52  
 Azathioprine 409

### Bacteriology

haemophilus influenzae meningitis ampicillin 211  
 phagocytosis in rickets 288  
 Salmonella Thompson neonatal meningitis 427

### Bilirubin

excretion newborn infants 148 153  
 oestrogen influence of 597  
 phenobarbitone influence of 596 681  
 pregnanolol hyperbilirubinaemia 451

### Blood

acute leukaemia mediastinal infiltrate 90  
 beta thalassaemia diagnosis in newborns 523  
 bleeding disorders glycogen storage disease 527  
 coagulation cyanotic heart disease 551  
 erythroblastosis late prognosis 112  
 erythroblasts immunosuppressive therapy 44  
 fibrinogen newborns with resp distress syndrome 2  
 fibrinolysis foetus 369  
 fibrinolysis newborns 33 281  
 haemolytic uraemic syndrome 347  
 haptoglobin in malignant disease 381  
 hemoperin haptoglobin hemolytic diseases 121  
 intravascular coagulation 664  
 leukemia cerebromeningeal cytosine arabinoside 164  
 myeloproliferative disease chromosomal anomalies 591  
 normal values 49  
 Rh immunization prevention of 601

### Cardiovascular system

atrial flutter congenital 587  
 chromosomal conditions congenital heart disease 63  
 congenital heart disease coagulation in 551  
 congenital heart disease intellectual development 169  
 mediastinal leukaemic infiltrate 90  
 portal hypertension 472

### Chromosomes

fatal granulomatous disease 403  
 congenital heart disease 63  
 Cri du Chat syndrome hereditary 348  
 myeloproliferative disease 591  
 XXXOY chromosome anomaly 706  
 Cri du Chat syndrome 348  
 Crohn's disease 473  
 Cystathioninuria 80  
 Cystinosis 613

### Ectodermal dysplasia 94

### Endocrine organs

diabetes insipidus growth hormone in 343  
 gastric mucosal changes endocrine disorders 443  
 hyperparathyroidism primary 249  
 hypoparathyroidism familial hypomagnesaemia 715  
 hypothyroidism ataxic syndrome in 323  
 hypothyroidism testicular enlargement 317  
 Kallman's syndrome  
 testosterone excretion congenital adrenal hyperplasia  
 vasopressin growth hormone response to 343

### Enzymes

acid phosphatase synovial fluid 205  
 dipeptidases 443 456 621  
 enterokinases 456 457 458  
 galactokinase 669  
 sucrase isomaltase 460

### Epidermolysis bullosa 101

### Fatal granulomatous disease 403

### Foetus

acoustic stimulation response to 599  
 electrolytes plasma red cells 192 598  
 ethanol effect on uterine contractility 597  
 fibrinolysis 369 598  
 salicylate damage: mucosa 517  
 tracheobronchial fluid 112

### Fructosemia

tyrosine metabolism 141 227 432

## Thalassemia 53

### Therapy

- asperillus ha myophilus influenzae meningitis 211
- azathioprine 409
- cytotoxic arabinoside cerebromeningeal leukemia 164
- zinc-sulfate 44

## Torsades 103 104

### Tumor

- gangliogliomas endocrine tumors 103
- hepatoblastoma hepatocarcinoma 107
- neuroblastoma classification 102
- neuroblastoma cystic degeneration 80
- neuroblastoma prolonged survival 711
- teratoma, intracranial 107

### Urology

- bladder function myelomeningocele 491
- bladder stone disease 177 385
- ectopic vesicle craniocoele 469

## Urology transplantation 471

- anastomosis cinematography 470
- renal function recurrent infection 605
- urethral reflux 469
- urethra malformations of 469
- urinary flow 470
- urostomy collection of urine 468

### Vaccination

- epidemic influenza 440
- measles 441
- measles vaccination maple syrup urine disease 2
- polio 441 536
- smallpox 442

### Virology

- adenovirus type 7 meningitis 310
- cytomegalovirus infections 303

### Vitamins

- B<sub>12</sub>-dependency methylmalonic acidemia 88

- hyperlipidaemia glycogen storage disease 701
- Lesch Nyhan disease 233 259
- lipid in newborns 348
- methionine transport 450
- methylmalonic aciduria 28 88
- phosphoglycerides red cells 637
- testosterone excretion 137
- Methodology**
  - biopsy cerebral cortex 108
  - capillary v. venous blood normal blood values 49
  - cardiography 600
  - collection of urine in urostomies 468
  - fine needle liver biopsy in storage diseases 293
  - parenteral feeding 472
  - spirometry 648
  - split function renal 1
- Methylmalonic aciduria**
  - amino acid pattern 28
  - vitamin B<sub>12</sub> dependency 88
- Mongolism**
  - somatic deviations 199
- Mucopolysaccharidoses** 539
- Nephrology**
  - creatinine in serum normal values 399
  - diuretics effect of in newborns 659
  - hemodialysis infants 221
  - immunosuppressive therapy 44
  - kidney transplantation 471
  - pyelonephritis childhood glomerular filtration rate 1
  - reabsorption of water 513
  - renal insufficiency sarcoidosis 582
- Nervous system**
  - ataxia in congenital hypothyroidism 323
  - ataxic diplegia cellular immunity 545
  - cerebral development 101 116
  - cerebral malformations thrombocytopenia 185
  - cerebromeningeal leukemia therapy 164
  - congenital teratoma intracranial 102
  - encephalopathy progressive 106
  - epidermolysis bullosa neuropathology in 101
  - head injuries sleep studies 565
  - hydranencephaly 105
  - intracranial hypertension benign 328
  - meningitis adenovirus type 7
  - myelomeningocele anal continence 491
  - myelomeningocele normal bladder function 487
  - poliodystrophus cerebri 100
  - p. euronemor cerebri 328
  - sleep 572
- Newborn infants**
  - amino acids plasma small for dates 19 21
  - beta thalassaemia diagnosis of 523
  - bilirubin excretion 148 153
  - body temperature anoxia rabbits 603
  - costochondral junctions perinatal death 105
  - dislocation of epiphyses 377
  - diuretics effect of 659
  - drug metabolism 596
  - duodenal obstruction 468
  - early feeding piglets 602
  - EEG in asphyxia
  - fatty acids anoxia rabbits 603
  - fibrinolysis 33 281
  - gamma globulins spina bifida 134
  - haptoglobin 121
  - hemopexin 121
  - hyaline membranes 208
  - hyponatremia following asphyxia 52
  - hypothermia lambs 600
  - inclusion body disease 227
  - infections 114 115
  - intracranial teratoma 102
  - lipid metabolism 348
  - lipolysis piglets 602
  - lipoproteins 349
  - malformations 297
  - meningitis 226 427
  - mongolism somatic deviations 199
  - oestrogen bilirubin level 597
  - osteomyelitis 227
  - parenteral feeding after surgery 472
  - perinatal mortality 601
  - phenobarbitone serum bilirubin 596 681
  - pneumonia 226
  - prognosis of pathological conditions 113
  - prolonged hyperbilirubinaemia 451
  - respiratory quotient metabolic rate 653
  - Rh immunization prevention of 601
  - serum lipids 445
  - skin temperature acid base balance 115
- Odontology**
  - fluoride ingestion bottle fed infants 424
- Osteogenesis imperfecta** 340
- Phenylketonuria**
  - renal transport of amino acids 41
- Premature infants**
  - intensive care of 353
- Respiratory distress syndrome** 208 281 497
- Respiratory tract**
  - centriolobular emphysema oxygen treatment 697
  - expiratory flow rates 648
  - hyaline membranes bronchial arteries 208
  - pathology postnatal period 497
  - respiratory distress syndrome fibrinogen in 281
  - respiratory distress syndrome fibrinolysis in 281
  - respiratory quotient newborns 653
- Rheumatoid arthritis**
  - synovial fluid acid phosphatase 205
- Rickets**
  - bone marrow 363
  - phagocytosis evaluation of 288
- Sarcoidosis hypercalcaemia renal function** 582
- Silver's syndrome** 435
- Skin**
  - epidermolysis bullosa 101
  - melanocytoma 538
  - nuchal naevi interscapular telangiectases 416
- Sleep** 565 572
- Social paediatrics**
  - anthropometry Ethiopian preschool children 157
  - infant mortality neonatal hospital units 112
  - mother and child welfare in Poland 110
  - social research in paediatrics 263
- Sudden unexpected death** 685

